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Main laboratory methods used for the isolation and identification of *Staphylococcus* spp.

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Summary

Introduction: Laboratory diagnosis related to clinical microbiology is highly complex, due to subjectivity when interpreting the results. Among bacterial pathogens, those linked to the *Staphylococcus* genus are relevant from a clinical and epidemiological point of view. **Aim:** To review the literature relative to the main laboratory methods used for the isolation and identification of microorganisms of the genus *Staphylococcus*. **Methodology:** The study was based on a literature search between 2000 and 2020, in the BVS, PubMed, Scielo, ScienceDirect and CAPES Periodical databases. Regarding its laboratory diagnosis, microscopy is used by the Gram method to verify the bacterium's morphology; novobiocin test, aiming to trace coagulase negative strains; use of biochemical tests, such as coagulase and catalase tests; identification through the use of selective and nonspecific culture media, such as blood agar (in which the hemolytic patterns of pathogens are observed), salty mannitol agar (specific to the species of *Staphylococcus aureus*) and DNAse agar (selective to *S. aureus* strains , *S. intermedius* and *S. hyicus*). **Results:** The *Staphylococcus* genus is of great clinical importance in the field of public health. Thus, the quality and speed of diagnosis are essential to promote measures to combat these pathogens, contributing to the well-being and survival of those affected.

Keywords: Microbiology, *Staphylococcus*, clinical laboratory techniques.

Resumen

Principales métodos de laboratorio utilizados para el aislamiento e identificación de *Staphylococcus* spp.

Introducción: el diagnóstico de laboratorio relacionado con la microbiología clínica es muy complejo, debido a la subjetividad al interpretar los resultados. Entre los patógenos bacterianos, los relacionados con el género *Staphylococcus* son relevantes desde un punto de vista clínico y epidemiológico. **Objetivo:** revisar la literatura relacionada con los principales métodos de laboratorio utilizados para el aislamiento e identificación de microorganismos del género *Staphylococcus*. **Metolodogía:** el estudio se basó en una búsqueda bibliográfica entre 2000 y 2020, en las bases de datos BVS, PubMed, Scielo, ScienceDirect y Periódicos CAPES. En cuanto a su diagnóstico de laboratorio, se utilizan la microscopía y el método de Gram para verificar la morfología de la bacteria; prueba de novobiocina, con el objetivo de rastrear cepas negativas de coagulasa; uso de pruebas bioquímicas, como las pruebas de coagulasa y catalasa; identificación mediante el uso de medios de cultivo selectivos e inespecíficos, como agar sangre (en el que se observan los patrones hemolíticos de los patógenos), agar manitol salado (específico para la especie de *Staphylococcus aureus*) y agar ADNse (selectivo para cepas de *Staphylococcus aureus*), *Staphylococcus intermedius* y *Staphylococcus hyicus*). **Resultados:** el género *Staphylococcus* es de gran importancia clínica en el campo de la salud pública. Por lo tanto, la calidad y la velocidad del diagnóstico son esenciales para promover medidas para combatir estos patógenos, contribuyendo al bienestar y la supervivencia de los afectados.

Palabras clave: Microbiología, *Staphylococcus*, técnicas de laboratorio clínico.

Resumo

Principais métodos laboratorais utilizados para o isolamento e identificação de *Staphylococcus* spp.

Introdução: o diagnóstico laboratorial relacionado à microbiologia clínica é de elevada complexidade, em virtude da subjetividade perante na interpretação dos resultados. Dentre os patógenos bacterianos, os vinculados ao gênero *Staphylococcus* são relevantes desde o ponto de vista clínico e epidemiológico. **Objetivo:** fazer uma revisão narrativa sobre os principais métodos laboratoriais utilizados para o isolamento e identificação de microrganismos do gênero *Staphylococcus*. **Metodologia:**

o estudo baseou-se em uma pesquisa na literatura entre o período de 2000 a 2020, nas bases de dados BVS, PubMed, Scielo, ScienceDirect e Periódico CAPES. Quanto ao seu diagnóstico laboratorial utiliza-se a microscopia, através do método de Gram, para verificação da morfologia da bactéria; teste da novobiocina, objetivando o rastrearmento de cepas coagulase negativa; uso de provas bioquímicas, como os testes de coagulase e catalase; identificação por meio da utilização de meios de cultura seletivos e inespecíficos, como o agar sangue (em que se observa os padrões hemolíticos dos patógenos), agar manitol salgado (específico a espécie de *Staphylococcus aureus*) e agar ADNse (seletivo as estirpes de *Staphylococcus aureus*, *Staphylococcus intermedius* e *Staphylococcus hyicus*). **Resultados:** o gênero *Staphylococcus* é de grande importância clínica no âmbito da saúde pública. Assim, a qualidade e rapidez do diagnóstico são imprescindíveis para promover medidas de combate a esses patógenos, contribuindo para o bem-estar e sobrevida dos acometidos.

Palavras-chave: Microbiologia, *Staphylococcus*, técnicas de laboratório clínico.

Introduction

Laboratory diagnosis related to clinical microbiology is highly complex, due to subjectivity when the results are interpreted. Even with the development of more modern and specific methods, through the incorporation of genomics and proteomics in microbiology and laboratory automation, the interpretation of the results is still linked to the quality of the samples for analysis. Therefore, the data need to be accurate and clinically relevant, which is why laboratories request that any microbiological samples be correctly chosen, collected and transported in order to improve their analysis and interpretation [1].

Initially, the procedure that must be performed for a microbiological diagnosis is the collection of biological material, followed by transport and analysis in the laboratory, which can be done through the use of dyeing methods, biochemical tests, and culture media.

It is recommended by health organizations that the isolation of the microorganism occurs from samples collected in the body region where the infection is occurring, since in this place there is a greater concentration of microorganisms that are causing the infectious process [2]. It is also important to emphasize the need to perform antisepsis of the site before collection and to use sterile instruments in the procedure, in order to reduce contamination by other microorganisms. In addition, it should always be recommended that the collection be performed in the most acute phase of the disease, as it is likely that the patient is not yet using antimicrobial drugs.

The isolation of bacteria allows the responsible professional to observe characteristics using microscopy and dyeing methods. Culture, in turn, provides an analysis of the sensitivity of pathogens to chemotherapeutic drugs, in addition to contributing to differentiation between genera and species (selective and differential means). In relation to gene sequencing tests and proteomic studies, it is necessary to obtain a pure culture, which makes it possible to verify the invasive potential of the pathogen, mechanisms of virulence and resistance to antibacterial drugs [3].

When compared to clinical diagnosis, laboratory testing is preferable, since the signs and symptoms seen in illnesses are generally inconclusive and similar to other diseases. In addition, early diagnosis, in the case of bacterial infections, is essential, enabling the faster initiation of recommended therapies, avoiding possible complications to the patient [4].

Among bacterial pathogens, those linked to the *Staphylococcus* genus are relevant from a clinical and epidemiological point of view. They belong to the Microccaceae family, which can be classified as Gram-positive, immobile, non-spore producing cocci, positive catalase and facultative anaerobes. These microorganisms are part of the normal microbiota of healthy individuals, thus being located on the skin and mucous membranes, however they can also be found in food, sewage, air and feces. They present several forms that vary from isolated, in pairs, in short chain and grouped in an irregular way. They are considered mesophilic agents with growth between 7-47.8 ºC and can synthesize heat-resistant enterotoxins. In addition, these microorganisms proliferate in media consisting of relative concentrations up to 15% sodium chloride (NaCl) [5-7].

Staphylococus spp. they can be divided into two groups: those with positive coagulase, which is comprised of four species (*S. aureus*, *S. intermedius*, *S. hycuse* and *S. delphinie*) and those with negative coagulase, corresponding to more than ten species. Both groups are of clinical interest because they cause food poisoning and healthcare-related infections [8-11].

Among the species included in the genus, *Staphylococcus aureus* is the most clinically important, its incidence varies between 10 to 30 cases per 100 000 people per year, in which the most affected ages are at the extremes of life (children and the elderly), being the black population was shown to be prevalente [12, 13].

Due to the negative impact on public health caused by microorganisms present in the genus *Staphylococcus,* it is necessary to carry out an effective, quality, and rapid diagnosis, aiming at an appropriate treatment and cure of the patient. However, there is little scientific literature on this subject. In view of this, the purpose of this study is to

conduct a narrative review on the main laboratory methods used for the isolation and identification of microorganisms of the genus *Staphylococcus*.

Materials and methods

This research is a review of literature of the narrative type, in which there was a search for articles, books, dissertations and theses in the databases: BVS (Regional Health Portal , PubMed, SciELO, ScienceDirect and Periodical CAPES, with the following associated descriptors: *Staphylococcus* AND diagnóstico, *Staphylococcus* AND diagnóstico laboratorial, *Staphylococcus* AND microscopia, *Staphylococcus* AND coloração de Gram, *Staphylococcus* AND teste da catalase, *Staphylococcus* AND teste da coagulase, *Staphylococcus* AND meios de cultura, *Staphylococcus*AND agar sangue, *Staphylococcus* AND agar manitol salgado, *Staphylococcus* AND agar DNAse, *Staphylococcus* AND teste da novobiocina, MRSA AND diagnóstico, VISA AND diagnóstico, VRSA AND diagnóstico, *Staphylococcus* AND diagnosis, *Staphylococcus* AND laboratory diagnosis, *Staphylococcus* AND microscopy, *Staphylococcus* AND Gram coloring, *Staphylococcus* AND catalase test, *Staphylococcus* AND coagulase test, *Staphylococcus* AND culture media, *Staphylococcus* AND blood agar, *Staphylococcus* AND mannitol salt agar, *Staphylococcus* AND DNAse agar, *Staphylococcus* AND novobiocin test, MRSA AND diagnosis, VISA AND diagnosis, VRSA AND diagnosis .

Articles published between 2000 and 2020 were included, prioritizing the most recent publications that addressed the proposed theme which presented information on concepts and diagnosis of the *Staphylococcus* genus and that were published in English or Portuguese. Studies that did not deal with diagnoses of the *Staphylococcus* genus, which brought information about other bacterial genera or that were not available in full, they were excluded.

Literature revision

Staphylococcus are responsible for the appearance of several types of diseases, however they have been associated, mainly with skin infections such as impetigo, folliculitis, boils, carbuncles and in some situations they can even gain blood flow causing septicemia [12].

The collection of purulent materials must be performed after previous cleaning of the lesion, wound or abscess margins using a solution of aqueous Povidone-iodine (PVP-I) and saline solution (half/half). Then, the actual collection is done by aspirating with a syringe and needle, in order to collect the purulent material present in the innermost region of the wound avoiding contamination, or through swabs. Finally, the material is

transported to the laboratory, in which the use of means of transport such as Amies or Stuart is recommended, contributing to the preservation of the sample and viability of the microorganism [14].

Microscopy

Microscopy plays an important role in the biological and medical sciences, contributing to great progress in understanding the systems of living beings and diseases. Such advances are provided due to their property related to optical resolution, since it makes it possible to determine structures with very small sizes. Improvements in this area aim at increasing the optical resolution and, consequently, the quality of the images viewed through the microscope [15].

In microbiology, this method allows the health professional to describe the morphology of the pathogen, measure the number of microorganisms present per field of view and determine the type of motility of the bacteria [16]. In some circumstances it is essential to perform staining techniques, to visualize the shape and bacterial arrangement. As an example, for *Staphylococcus* spp., Gram stain is used, in which the presence of coconuts that can be joined together is observed, resembling *grape bunches* [12].

Gram stain

Gram staining comprises the tintorial method of greatest use and relevance in microbiology, which aims to classify pathogens by their size, shape, cell structure and color. Based on this methodology, bacteria can be organized into two groups: Gram-positive and Gram-negative. Gram-positives are colored purple and Gram-negatives are colored red [17, 18].

The dyes used are violet crystal (VC) and safranin. VC is a cationic dye belonging to the chemical group of triphenylmethanes, which is sold in concentrations between 1-2%, being responsible for attributing the characteristic color to Gram-positive organisms. Safranin, on the other hand, is a cationic dye that makes it part of the group of triarylpyrazines. It has a color spectrum distinct from the VC, giving Gram-negative pigmentation to its particular pigmentation. In addition, both are hydrophilic [19-22].

At first, a smear is prepared with the bacterial strain on the slide intended for microscopy, in which the smear is fixed using heat or some chemical method. Then, the VC is dripped on the slide, allowing it to react for 1 min. Right after, washing with running water is carried out to remove the excess of VC and then the lugol is added for 1 min which will have the function of allowing the fixation of the primary dye, followed by washing.

Afterwards, a bleach called alcohol-acetone is used for 10 seconds which is removed with water after the stipulated time. Gram-positive bacteria have the ability to retain VC, because their cell wall is quite thick with peptidoglycan, which forms a mesh. This mesh when suffering the action of alcohol-acetone and dehydrates, consequently, the network is blocked, preventing the dye from leaving the wall. Gram-negatives, in turn, have a cell wall with a thin layer of peptidoglycan, so their water loss will not be enough to provide the mesh closure, so the dye is not retained. In addition, Gram-negative microorganisms have an external cell membrane rich in lipids, in which upon contact with the organic solvent, they are solubilized, resulting in the elimination of adhered VC. Thus, Gram-positive stains purple due to the VC whereas Gram-negative ones are transparent, since the VC will not be retained in this type of bacteria due to the bleach and its small amount of peptideoglycans.

Finally, the safranin is dripped, allowing it to react for 1 min, followed by washing. Then, the slanted slide is placed to remove excess dye and allow drying. Thus, as the Gram-negatives were without the presence of dye they will be able to retain the safranin, staining red or pink, while the Gram-positives as they already had the VC used in their structures, will not have the ability to retain another type coloring agent, remaining purple. In addition, the slide is analyzed with immersion oil in the optical microscope in the 100x objective [19, 23]. This methodology can be seen in figure 1. Therefore, microbes belonging to the *Staphylococcus* genus are classified as Gram-positive cocci, so they are stained purple [24].

Bacterial identification tests

Catalase test

Hydrogen peroxide (H_2O_2) is a reactive oxygen species synthesized by human metabolism and which is indispensable for the leukocyte's antimicrobial activity. However, some bacteria produce an enzyme called catalase that works as a way of protecting the microbe against this mode of action of the immune system, as it will promote the degradation of H_2O_2 in water (H_2O) and oxygen (O_2) [25].

The catalase test consists of a qualitative method for identifying this enzyme in the bacterial species analyzed, aiming at distinguishing between the genera *Staphylococcus* and *Streptococcus*, since among these genera only *Staphylococcus* will be able to produce it. From a smear of the strain on a glass slide, a drop of 3% H_2O_2 is dripped, and subsequently, the formation or not of bubbles originating in the microbial colony is verified [26]. Such methodology can be seen in figure 2.

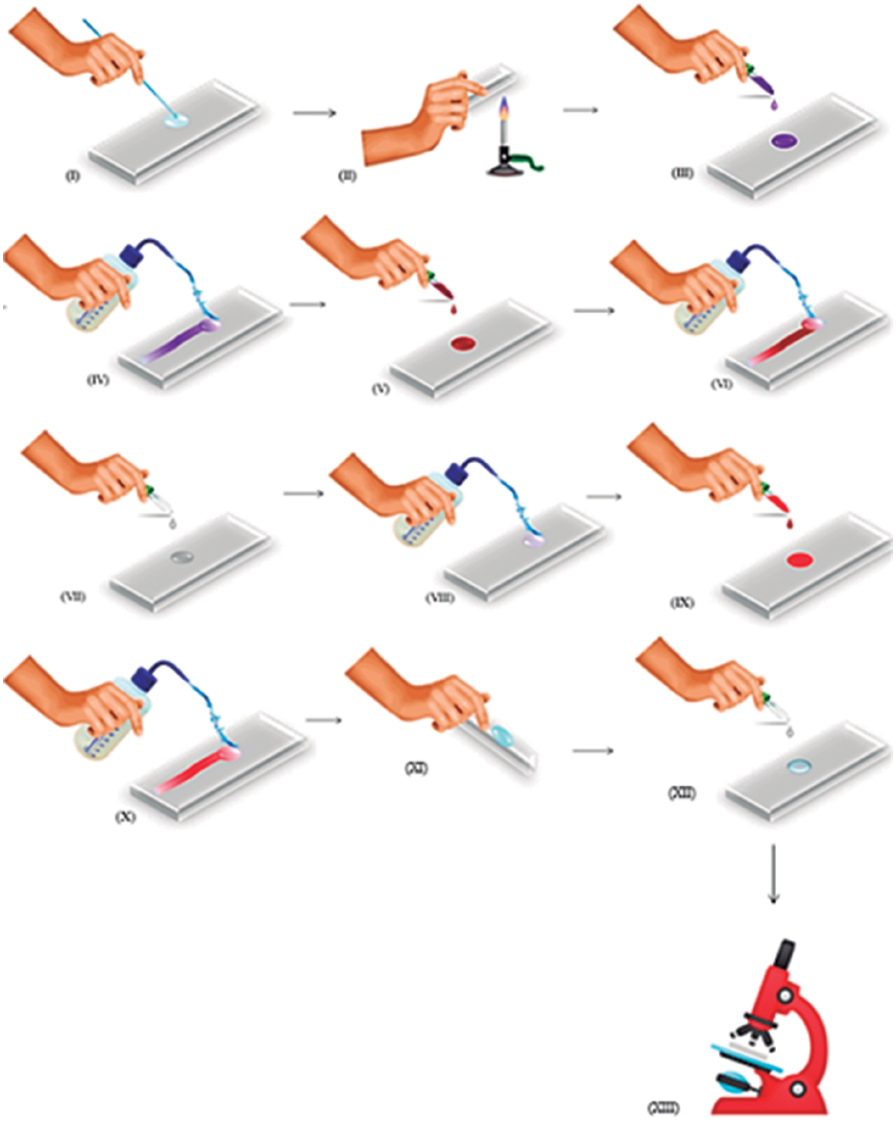

Figure 1. Gram staining steps. (I) Preparation of the bacterial smear. (II) Smear fixed through the heat emitted by the flame of the Busen burner. (III) Dripping the CV until it covers the entire smear, leaving it to rest for 1 min. (IV) Blade washing. (V) Drip from the lugol until it covers the entire smear, leaving it to rest for 1 min. (VI) Blade washing. (VII) Dripping of the bleach until it covers the entire smear, leaving it to rest for 10 seconds or less. (VIII) Blade washing. (IX) Dripping the safranin until it covers the entire smear, leaving it to rest for 1 min. (X) Blade washing. (XI) Inclination of the blade. (XII) Dripping immersion oil on the slide. (XIII) Observation of the slide under the microscope.

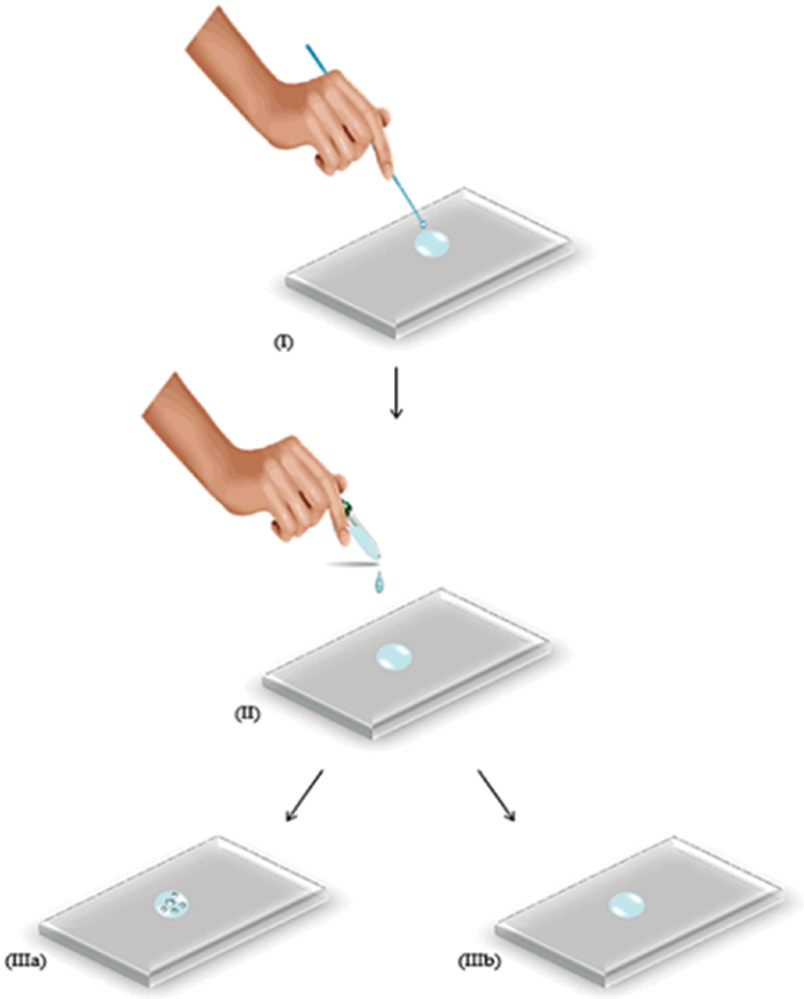

Figure 2. Test steps to identify the catalase enzyme. (I) Smear of the bacterial strain. (II) 3% H_2O_2 dripping. (IIIa) Blistering in the bacterial smear. (IIIb) There was no bubble formation in the bacterial smear.

In the test, the visible bubbling of the smear is triggered by the release of O_2 (product of the reaction) to the environment, indicating that the pathogen possessed the ability to produce the enzyme, being thus classified as catalase-positive. However, if bubbles do not occur, the bacterium is considered catalase-negative [27]. Thus, the genus *Staphylococcus* is categorized as catalase-positive and *Streptococcus* spp. it is classified as catalase-negative [28, 29].

Coagulase test

Coagulase is an extracellular enzyme synthesized by some species of *Staphylococcus*, which is considered a virulence factor of the microorganism. In contact with blood plasma, this protein acts by reacting prothrombin, creating a complex that will be responsible for providing the conversion of fibrinogen into fibrin, coagulating the plasma [30].

The coagulase identification method allows the recognition of species of the genus *Staphylococcus* spp. In addition, this test is characterized by being fast and inexpensive, due to its speed in making and using few materials [31].

This test consists of inoculating a suspension of the bacterial strain in rabbit plasma in a test tube, which will be incubated in a bacteriological incubator at 37 ºC, for 24 h. Over this time period, it will be observed whether or not there is the formation of a clot in the plasma, in which the presence of coagulation will indicate a positive result and the opposite, negative [32, 33]. Such methodology is represented in figure 3.

Another way of carrying out the coagulase test, occurs through the use of a slide in which the blood plasma of the rabbit drips into the microbiological suspension, which are observed to form or not the clot in a short period of time. However, the realization of the methodology using the test tube is more sensitive and reliable [34].

Thus, the strains of *Staphylococcus aureus*, *Staphylococcus intermedius*, *Staphylococcus delphini* e *Staphylococcus hyicus* are coagulase-positive, however, the other species of the genus are classified as negative coagulase, which can be highlighted, because of their clinical importance, since they affect most frequently immunodeficient patients, the following microorganisms: *Staphylococcus epidermidis*, *Staphylococcus hominis*, *Staphylococcus capitis*, *Staphylococcus haemolyticus*, *Staphylococcus simulans*, *Staphylococcus xylosus*, *Staphylococcus warneri*, *Staphylococcus cohnii* and *Staphylococcus carnosus* [35, 36].

Culture media

The culture media are used to identify microorganisms, with a view to promoting an environment conducive to the multiplication and survival of bacteria and fungi, due to supplying their needs to essential substances such as carbon, nitrogen, minerals, water, factors of growth and vitamins [37].

Blood agar

Blood agar consists of a culture medium widely used in clinical microbiology laboratories, being easy to prepare and low cost [38]. This culture medium consists of 5% blood from some mammal, usually that of the sheep. In addition, it allows the identification

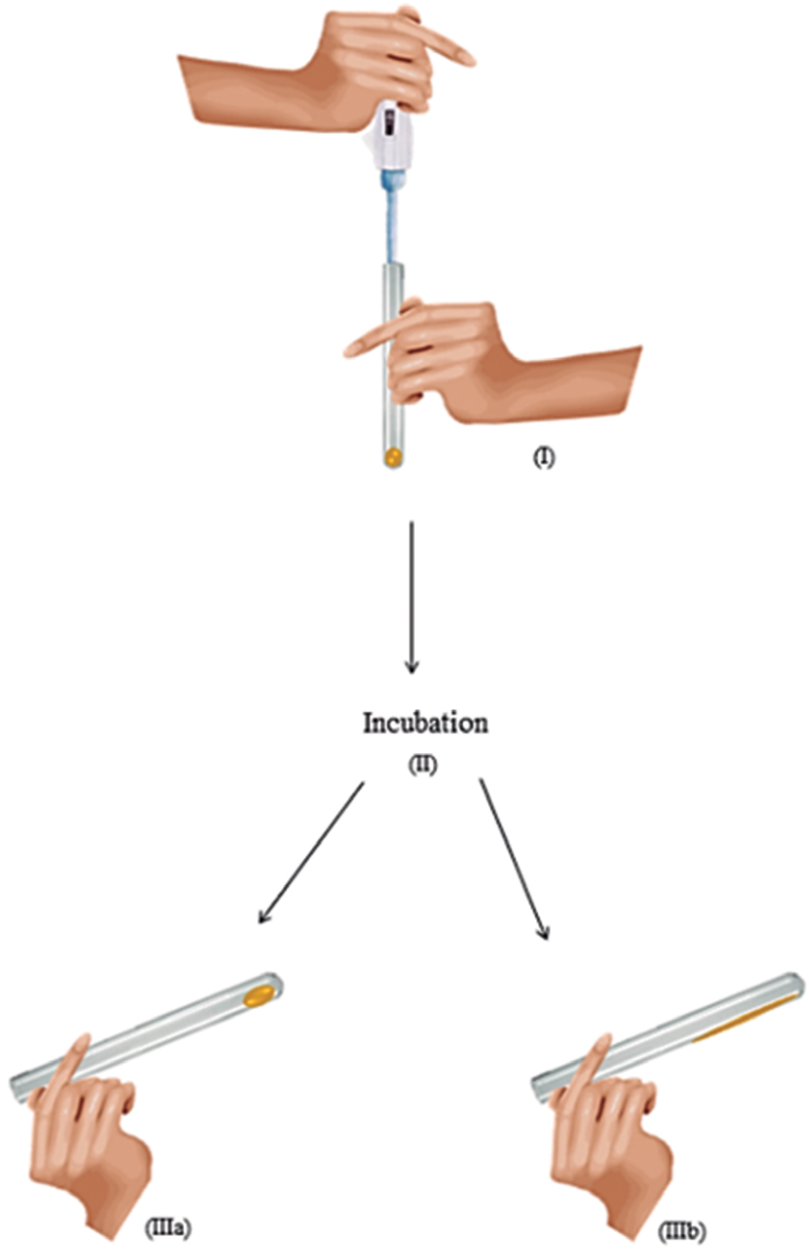

Figure 3. Stages of the coagulase test. (I) Rabbit plasma is added to the bacterial suspension. (II) Incubation of the material in a bacteriological incubator. (IIIa) There was formation of the clot. (IIIb) There was no clot formation.

and isolation of several bacteria, therefore they are nonspecific, however, the distinction of species and genera is possible, according to the hemolytic standards of each strain [39].

In this sense, colonies of the genus *Staphylococcus* on blood agar, are checked with a white or yellow tint, creamy, opaque, and convex. Some species, such as *Staphylococcus aureus*, form a transparent halo close to the places found in the culture medium, as they are capable of providing a β-hemolysis (total hemolysis) [40, 41]. However, the difference in hemolytic patterns cannot be used as the sole diagnostic criterion, and there must be the use of staining methods, biochemical tests, and selective means to contribute to the correct identification of any and all microorganisms.

Salted Mannitol Agar

Salted mannitol agar is a selective culture medium, that is, specific to certain bacteria, since it is composed of 7.5% sodium chloride (NaCl) and, therefore, selects pathogens that have the ability to survive high concentrations of salt. In addition, it also contains a glycide (mannose) and a pH indicator called phenol red. Microbial growth occurs through the fermentation of this sugar, which will promote a change in the pH of the culture medium, chemical changes in the indicator and a consequent change in color from red to yellow. Thus, strains of *Staphylococcus aureus* (since they have the ability to ferment mannose and survive high concentrations of NaCl) will form yellow colonies, however the other species of the genus (salt tolerant) will result in reduced pink or red colonies, representing an absence of change in shade of salty mannitol agar [42-44].

DNAse agar

DNAse agar is composed of deoxyribonucleic acid (DNA), which will serve as a substrate for deoxyribonuclease enzymes (DNAse) that can be produced by some microorganisms. DNAse will act by depolymerizing the DNA, resulting in the formation of a transparent halo in the culture medium that can be visualized around the colonies. Strains of *Staphylococcus aureus*, *Staphylococcus intermedius* and *Staphylococcus hyicus*, are considered DNAse positive. In the other species of the genus there is no halo formation, therefore they are negative to the test [45-48].

As an alternative method, 0.1% orthotoluidine blue dye can be added or purchased included in the DNA agar. This addition to the medium has the advantage of providing a more comprehensive reading, due to its ease when viewing the exam, as positive samples will pigment with pink around the enzyme-producing colonies [49].

Novobiocin test

Novobiocin, derived from coumarins, is an antibacterial drug used in humans for the treatment of infections caused by Gram-positive bacteria. This drug acts on the topoisoimerase B subunits of microorganisms, called gyrase B, affecting the unfolding of DNA, and preventing its replication [50, 51].

The novabiocin test aims to screen for negative coagulase strains, by performing the raising in Petri dishes filled with Agar Müller Hinton medium, plus a novobiocin disc containing 5 μg. The resistant strains will present an inhibition zone between 6-12 mm, on the other hand, sensitive species will present values greater than 16 mm [52].

Thus, all strains of *Staphylococcus saprophyticusis* are resistant to novobiocin. The rest of the species comprising the genus, usually in the laboratory routine, are sensitive to the active substance [53].

Techniques used to assess microbial resistance

Broth dilution

Broth dilution is a method applied to determine the Minimum Inhibitory Concentration (MIC), which is the lowest concentration necessary to inhibit the visible growth of bacteria and fungi.

In this technique, there is an analysis of the relationship between the growth of the microorganism in the broth and the concentration of the test drug, in which the growth of the bacterium or fungus is observed by turbidity of the liquid medium. This assay enables quantitative results to the clinical analyst and no interferences promoted by the microbe's development kinetics are observed. As a sterility control, culture broth without substances or inoculum is used in parallel to the test. As for the viability control, culture broth and inoculum are used, without test substances [54]. In addition, dilution in broth can be done by means of macrodilution or microdilution.

The macrodilution corresponds to the experiment that is performed using test tubes, in which there may be variations from 1 to 10 mL in the volume of the culture medium and test substance. However, there are some disadvantages: more waste, requires more space to couple the tubes and has a limited number of replicas [54, 55]. In microdilution, in turn, microplates consisting of 96 wells are used. In each well, 100 μL of the most appropriate liquid culture medium is added and, immediately after, a serial dilution of 2 is performed with the tested drug, allowing the obtaining of different concentrations of the tested drug. In this methodology, several advantages are evidenced,

among them, the use of small amounts of culture medium and drugs, good precision, and sensitivity, however it is not so exact [7, 56].

Agar dilution

Agar dilution is a test characterized by the insertion of the analyzed substances in different concentrations in a solid culture medium. The solutions are deposited in individual Petri dishes, where each dish corresponds to a single concentration of the drug. Thus, the bacterial strains are introduced to the surface of the culture medium with the aid of a multi-inoculator, in which they distribute from 1 to 3 μL of the inoculum [57].

Etest®

The Etest® (AB Biodisk, Solna, Sweden) corresponds to a commercially acquired plastic tape, through which it is soaked in increasing concentrations of an antimicrobial. To perform the test, the microorganism is seeded on the surface of a solid culture medium. After 15 min, the tapes are distributed in a solid medium. After the incubation period, the MIC is determined visually. This method is quite advantageous in the routine of a clinical analysis laboratory, since it is versatile and has a good consonance with gold standard methodologies, however it has high cost which limits the use [58-60].

Disk diffusion (Kirby-Bauer)

The disk diffusion method is simple, reliable, practical, and inexpensive. This test consists of the dissemination of the drug contained in a filter paper disc in a solid culture medium. As a result, it will promote the formation of a transparent inhibition halo, indicating the non-growth of the bacterial colony, whose size is inversely proportional to MIC and millimeters (mm) is used as the unit of measurement [55, 61].

However, in this laboratory procedure some disadvantages are evidenced, such as the complexity existing in the interpretation of the diagnosis of anaerobic microorganisms, the need for nutritional supplementation of the culture media and the presence of complications related to the analysis of the sensitivity profiles to antimicrobial drugs that are not distributed correctly on the agar [61].

Automated methods

The automated methods use three types of equipment, commercially available, to carry out antimicrobial sensitivity tests, which are VITEK GPI® (bioMerieux®), Phoenix™ (BD Biosciences) and Microscan® (Dade Behring). These systems make it possible to perform susceptibility tests more quickly, being able to perform Gram identification and evaluate the sensitivity or resistance of each antimicrobial, thus being semi-quantitative. However, they are expensive, do not allow an MIC to be so accurate (with the

exception of Phoenix™, because it has the ability to perform serial dilution) and cannot perform the identification of VRSA strains [58].

Drugs used to determine *Staphylococcus aureus* resistance

Methicillin sensitivity test

Methicillin corresponds to a synthetic antimicrobial drug, belonging to the family of β-lactams. In clinical practice, this substance was commonly used in the therapy of infections caused by *Staphylococcus aureus*, which acts by adhering to penicillin-binding proteins (PBP's), making it impossible to synthesize the bacterium's cell wall and, consequently, allowing the lysis of the microorganism [62].

The methicillin sensitivity test aims to screen methicillin-resistant strains of *Staphylococcus aureus* (MRSA). Regarding its performance, several tests can be used, among them, the MIC determinations (Etest®, macrodilution or microdilution) and disk diffusion stand out. In addition, latex agglutination, chromogenic agar, automated methods, rapid screening methods and molecular approaches can be performed [58].

The mechanism of resistance to methicillin results from the coding of new genes, mecA or mecC, in which they are integrated with a mobile genetic element, the mec staphylococcal chromosome cassette (SCCmec), which can be identified in MRSA isolates. In addition, resistance is also checked, most drugs included in the β-lactam class [63].

Infections caused by MRSA strains are classified as emergent, due to the difficulties present in diagnosis and therapy, aimed at decreasing the rates of morbidity and mortality, thus being quite common in hospitals [64].

As for its epidemiology, it is constantly changing, which its resistance profiles and clones vary between countries and regions. Patients at higher risk for infection, in turn, are equivalent to the immunosuppressed, patients with chronic diseases, patients who use catheters, individuals exposed to people with this bacterium, the elderly and those with a history of prolonged hospitalizations [65].

Vancomycin sensitivity test

Vancomycin is an antibacterial drug that belongs to the pharmacological class of glycopeptides, which is recommended and used in therapies of resistant Gram-positive cocci. This drug acts on the cell wall of these bacteria preventing the synthesis of peptideoglycan [66, 67].

The mechanism of resistance to vancomycin is related to the displacement of a plasmid constituted by the transposon of the vanA gene, Tn1546, resulting from vancomycin-resistant

Enterococcus faecalis, in which it was integrated with the plasmid of the MRSA strain. The residual part of the enterococcal plasmid has been lost, while the staphylococcal region has the potential to be expressed and transmitted to other strains [68].

The vancomycin sensitivity test aims to screen strains of resistant *Staphylococcus aureus* or of lower sensitivity to vancomycin, in which depending on the degree of resistance, they are called Vancomycin-intermediate *Staphylococcus aureus* (VISA) and vancomycin-resistant *Staphylococcus aureus* (VRSA) [69].

When compared to VRSA strains, VISA strains are more prevalent, due to a lower number of microorganisms with reduced sensitivity and a variety of methodologies for their identification. Therefore, its prevalence changes according to the analyzed population, geographic region and hospitals [70].

Currently, the gold standard methodology used to identify these microorganisms is associated with tests that allow the determination of MIC. However, not all methods can be used, therefore, for VISA strains, a screening test, Etest®, dilution in broth and in agar are used. On the other hand, for VRSA strains the diffusion method and the same assays mentioned are used for the strains of VISA [58].

To perform the screening test, the suspected microorganism is inoculated into plates containing brain and heart infusion agar (BHI) with vancomycin 6 mg / mL, in addition to 10 μL of a standardized bacterial suspension from the 0.5 McFarland scale. Then, they are incubated for 24 h in a bacteriological oven, which at the end, the formation of bacterial colonies is verified, so the growth of more than one would represent a positive result and, therefore, resistance [55].

Table 1 shows the MIC and diameters of inhibition halos related to the sensitivity profile of *Staphylococcus aureus*, to the drugs methicillin and vancomycin.

Table 1. *Staphylococcus aureus* sensitivity profile to the drugs methicillin and vancomycin.

Drug	Microorganism	Inhibition halo diameter (±mm)			MIC (μg/mL)		
		S	I	R	S	I	R
Methicillin	*S. aureus*	≥14	10-13	≤9	≤8	-	≥16
Vancomycin	*S. aureus*	-	-	-	≤2	4-8	≥16

Source: Adapted from Zurita *et al.* [58].

The *Staphylococcus aureus* drug resistance reflects negatively on the infected patient, since it hinders pharmacotherapy and contributes to a poor prognosis. Therefore, a fast and safe diagnosis allows a more rational pharmacological treatment and a greater probability of favorable clinical outcomes.

Conclusions

The *Staphylococcus* genus comprises pathogens of great clinical importance in the field of public health. Thus, the quality and speed of diagnosis are essential to promote measures to combat these pathogens, contributing to the well-being and survival of those affected.

Its species are characterized by being Gram-positive and catalase positive. Regarding the coagulase test, species of this genus can be divided into two groups: positive coagulase and negative coagulase. Regarding the culture media used to identify the strains, salted mannitol agar and DNAse, are selective means, generally used in the laboratory routine to identify *Staphylococcus aureus*. Blood agar, in turn, is a means used for the growth of any bacterial colonies, which also allows the identification of hemolysis patterns. As for the novobiocin test, it is applied in the screening of *Staphylococcus saprophyticusis*, as it is the only one of the genus resistant to this drug.

In addition, MRSA and VRSA strains are characterized by having MIC equivalent to values greater than or equal to 16 μg/mL. VISA, in turn, has MIC between 4-8 μg/mL. Regarding the MRSA strain, more specifically, the diameter of the inhibition zone is less than or equal to 9 mm.

Since the microbiological diagnosis can be pointed out as a present difficulty among health professionals, this research may serve to improve these professionals and to encourage other research that has similar focuses.

Disclosure statement

No potential conflict of interest was reported by the authors.

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