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STUDY OF MICROBIOLOGY (STAPHYLOCOCCUS AUREUS) AND MOLECULAR BIOLOGY CLINICAL ISOLATES OF BACTERIA TO ANTIBIOTICS RESISTANCE

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Abstract
There are several resistance mechanisms found in Staphylococcus, the most common being resistance to macrolides, aminoglycosides, beta-lactams and glycopeptides. Directly linked to the hemostasis phase is the inflammation phase, which is characterized by the presence of inflammatory cells in the scar tissue. The localized release of histamine, serotonin and bradykinin causes vasodilation with a consequent increase in blood volume resulting in the clinical manifestations of heat and flushing. The important presence of prostaglandins in the region increases vascular permeability, in addition to favoring plasma exudation. They are responsible for promoting chemotaxis of leukocytes to the wound. Polymorphonuclear leukocytes together with neutrophils that are later replaced by macrophages are responsible for the phagocytic action of this process, helping to destroy bacteria, remove devitalized collagen and fibrin clots. Macrophages also activate fibroblasts and endothelial cells that will be needed in subsequent healing phases. Inflammatory cells undergo apoptosis as the process develops.

Introduction

The secretion of toxic 1 from toxic shock syndrome causes toxic shock syndrome, an acute and multisystem disease that can arise as a complication of any staphylococcal infection (skin, pharynx, vagina). Initially: white clinical picture that is confused with viral disease. Prodromes accompanied by initial dermatological involvement. Two weeks later there is a new erythematous and pruritic maculopapular rash that resolves with characteristic desquamation, especially of the palms and soles. There may be severe circulatory shock, with a mortality rate of around 7%, with vomiting, diarrhoea, muscle, liver, kidney and central nervous system impairment, respiratory failure, thrombocytopenic purpura being common. Diagnosis is clinical and treatment is performed with antibiotics and hydro electrolytic support (Butterfield, 2006).

1.1 Mechanism of resistance of Staphylococci to antimicrobials

The increasing number of bacteria increasingly resistant to antimicrobials stems from multiple factors that include; the widespread and often inadequate use of antimicrobials, extensive use as an animal growth promoting agent, since these antimicrobials act on the

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animal's intestinal microbiota, reducing competition for nutrients and reducing the production of metabolites that depress the growth of these animals. In addition, the growth in the number of regional and international trips increases the ease with which antimicrobial-resistant bacteria cross geographic barriers (Reygaert, 2013).

Antimicrobial resistance can occur through two major mechanisms: mutation in a chromosomal locus or by horizontal gene transfer, that is, by acquisition of resistance genes previously present in other microorganisms through mechanisms of transduction, transformation or conjugation. Staphylococcal resistance to almost all new classes of antibiotics is mediated almost exclusively by determinants acquired through horizontal DNA transfer. The acquisition of genetic material from another microorganism offers several advantages to Staphylococcus, the main one being the possibility of gaining a complete template of coding packages for resistance to multiple antibiotics (Pantosti et al., 2007).

Resistance to macrolides and lincosamides can occur by two mechanisms:

1. Modifications to the binding target on the ribosome, which confers cross-resistance to macrolides, lincosamides, and streptogramin B. This type of resistance is encoded by the ermA or ermC genes.
2. Active efflux, which confers resistance to macrolides and streptogramin B. It is encoded by the mrsA gene (Roberts et al., 2000).

With regard to aminoglycosides, the main resistance mechanism found in Staphylococci is drug inactivation by cellular aminoglycoside-modifying enzymes. These modifying enzymes are encoded by genes present in several different loci and previously characterized for Staphylococcus spp. The most clinically important enzymes encoded by these regions are acetyltransferase (AAC), adenyltransferase (ANT) and phosphotransferase (APH). Aminoglycosides, modified into amino groups by the AAC enzyme or into hydroxyl groups by the ANT or APH enzyme, lose the ability to bind to ribosomes and thus no longer inhibit protein synthesis in bacterial cells (Llano-Sotelo et al., 2007).

Methicillin-resistant Staphylococci are difficult to treat, usually because they are also resistant to most other available antibiotics. For these infections, the glycopeptides, vancomycin and more recently teicoplanin, are the drugs of choice. Due to the widespread use of vancomycin in clinical practice, S. aureus with reduced sensitivity to both vancomycin and other glycopeptides has emerged, these isolates are called S. aureus with intermediate sensitivity to vancomycin/glycopeptides (VISA/GISA) and resistant S. aureus to vancomycin/glycopeptides (VRSA/GRSA). It is believed that the acquisition of resistance to vancomycin in Staphylococci was due to the conjugative transfer of a plasmid containing the vanA operon into Enterococcus faecalis. This operon integrates the Tn1546 Transposon composed of vanR, vanS, vanX, vanY and vanH, present in a 120 Kb plasmid (Launay et al., 2006).

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1.2 Mechanism of resistance to beta-lactam antibiotics

Penicillin was the go to drug for treating severe S. aureus infections in the 1940s and 1950s, but resistance quickly emerged due to the spread of genetic elements that allowed for the production of extracellular penicillinase (class A beta-lactamase), an enzyme that hydrolyzes the amide bond of the beta-lactam ring in penicillin and ampicillin, rendering them inactive. Antibiotic resistance genes (erythromycin, fusidic acid, and aminoglycosides) are often co-located on the same plasmids that code for penicillinase production. Other resistance genes include those to disinfectants (quaternary ammonium compounds), acriflavine dyes, and heavy metals (lead, mercury, and cadmium) (King et al., 2017).

With regard to resistance to 2nd generation beta-lactam antibiotics, or semi-synthetic penicillins, it arises from mutations in PBPS that lead to a decrease in the binding affinity of the antibiotic to the Staphylococcus spp. acquired the mecA chromosomal gene, which encodes PBP2a, which makes it resistant to beta-lactams. This is a new PBP distinct from the others present in this genus and which decreases the affinity for most beta-lactam antibiotics. This protein confers a high standard of resistance to Staphylococcus, causing inhibition of all beta-lactams. In addition, it keeps active the synthesis of a stable peptidoglycan structure, which allows the growth and division of these microorganisms (Hamilton, 2017).

The mecA gene is included in a 30-60kb element that is a genomic island of resistance called Staphylococcal Cassette Chromosome mec (SCCmec), which may also contain other antimicrobial resistance genes. This gene is also present in methicillin-resistant coagulase-negative Staphylococci and is not present in strains susceptible to this antimicrobial. It is believed that this gene was acquired from species that are not closely related. According to the Clinical and Laboratory Standards Institute (CLSI), methicillin-resistant S. aureus may appear sensitive in vitro to other beta-lactams, combinations of beta-lactams and beta-lactamase inhibitors, carbapenems and cephalosporins, but these are ineffective in vivo. Two recombinase genes (ccrA and ccrB) mediate the element's site-specific integration/excision from the chromosome, and SCCmec is uniquely located on the staphylococcal chromosome within an unknown-function gene named orfX.

So far, eleven different types of SCCmec, which can be distinguished based on the structure of the mec complex, ccr genes and additional elements, have been described. SCCmec type I, II, III and VI are frequently associated with nosocomial infections. Types IV, V and VII, however, are more widely disseminated in strains of community origin (Zapun, 2008).

The first SCCmec (type I) was isolated in 1961, in the United Kingdom, from the MRSA strain NCTC10442, the SCCmec type II, was identified in the strain named N315, a MRSA isolated in Japan in 1982, while the SCCmec type III was identified in 1985 in New Zealand, in the MRSA strain named 82/2082. SCCmec type IV was already

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identified in the 90s, in the United States, in strains from community infections (Ito et al., 2001).

SCCmec type V was described in the strain of community origin called WIS (WBG8318), isolated in Australia in 1999. This element is structurally similar to SCCmectype IV, having only the mecA gene as a determinant of antimicrobial resistance.

SCCmec type VI was described in the strain HDE288, isolated in Portugal in 1996. It is also considered to have community origin and, despite showing similarity in size with SCCmec type IV, it differs in terms of the type of ccr presenting it (Berglund et al., 2005).

SCCmec type VII was described in the strain JCSC6082, of community origin, isolated in Sweden in 2002. SCCmec type VIII was described in a strain from an epidemic in Canada in 2003. SCCmec types IX and X were identified in a study carried out in China in strains from Thailand (type IX) and Canada (type X), both isolated in 2006. In addition to antimicrobial resistance, these SCCmec carry elements related to the detoxification of heavy metals such as cadmium, copper, zinc and arsenate. SCCmec type XI was identified by the Sanger Institute in the bovine strain of MRSA LGA251 (Ganamé, 2022).

According to experts, the SCC component gives the strain enhanced virulence and a higher chance of survival. This suggests that SCC not only transports genes for resistance to drugs, but also several helpful genes for coping with the harsh environment. Therefore, S. aureus is able to diversify its genome thanks to the accumulation of several SCC components with overlapping roles.

1.3 Epidemiology of infections caused by Staphylococcus spp. methicillin resistant

In the pre-antibiotic era, the mortality of patients infected with S. aureus was greater than 80%, and more than 70% of these developed metastatic infections. The introduction of penicillin into clinical practice in 1941 revolutionized the treatment of infections caused by gram-positive bacteria, mainly Staphylococci and streptococci, which improved such prognosis, with more than 94% of strains exhibiting sensitivity (Boucher, 2008).

This result, however, was short-lived. The use of penicillin quickly selected for strains that were resistant to the antibiotic as a result of beta-lactamase expression. In 1942, the first isolate of S. aureus resistant to penicillin emerged, with initial proliferation within the hospital environment and later in the community. In the 1950s, about 50% of isolated Staphylococci were resistant to penicillin. In response to the high rates of resistance to penicillin, methicillin, a semi-synthetic penicillin, appeared in 1959. Only two years later, in 1961, there were the first reports of strains isolated from methicillin-resistant S. aureus (MRSA) in the United Kingdom, which spread rapidly to other european countries, where there were outbreaks of nosocomial infections caused by this microorganism, also occurring reports of MRSA isolated in Japan, Australia and the United States (Yasmin, 2015).

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In several countries, MRSA has become an endemic nosocomial pathogen. These MRSA strains were restricted to the hospital environment for decades, causing infections only in individuals who had the classic risk factors for infections associated with health care, such as patients in the postoperative period, with chronic diseases, with a history of hospitalization, or even, people with prostheses or devices (Schaumburg et al. 2014).

Since the late 1980s and early 1990s, regular surveillance for MRSA has taken place in hospitals in the US and Europe. Even with the intensive surveillance program, the CDC estimates that 63% of staph isolates isolated in the US are MRSA. In 1974 these numbers showed only 2% of isolates, while in 1995 this rate rose to 22%. In contrast, countries with strict infection control practices, such as the Netherlands and Scandinavian countries, have maintained low rates of MRSA infection, even in hospitals (Schaumburg, et al. 2014).

CA-MRSA infections are defined as infections that occur in outpatients or that begin within the first 48 (fortyeight) hours of admission in patients without any of the following risk factors used to define HA-MRSA infection: hospitalization or recent surgery, prolonged antibiotic therapy, underlying chronic illness, central venous catheter or other devices, in long-term or institutionalized care. CA-MRSA strains differ in some respects from HA-MRSA strains. CA-MRSA strains are sensitive to a significant range of non-beta-lactam antibiotics, whereas HA-MRSA strains are typically multidrug-resistant. It is also observed that about 90% of CA-MRSA produce a toxin called Panton Valentine Toxin (PVL), capable of destroying human leukocytes and causing severe tissue damage (Cohen, 2007).

The dissemination of CA-MRSA strains, as well as their epidemic nature, are closely related to the changes that have occurred in the provision of health services, mainly in outpatient programs for the treatment of chronic diseases, such as hemodialysis, peritoneal dialysis, day hospital programs and long-stay asylums, etc. These programs favor the dissemination of clones in the community. Another factor related to the spread of CA-MRSA clones is the type of SCCmec found. The smaller size of SCCmec types IV to XI suggests greater ease of transmission between them compared to large SCCmec types I to III (Co, 2011).

In Iraq, there are high frequencies of isolation of S. aureus, as well as its strong relationship with nosocomial infections. Several studies have shown a prevalence of 40 to 80% of isolation of MRSA strains in Iraqi hospitals. It is also verified that data from the Antimicrobial Surveillance Program indicate that MRSA corresponds to 31% of the cause of nosocomial and community infections, being considered the most common among the most prevalent pathogens. The first report of disease caused by CA-MRSA in the country occurred in 2008, by a sample of SCCmec type IV and positive PVL (Al-Mathkhury, 2013).

Antibiotic resistance is a worldwide concern, as it compromises the successful treatment of infections. The correct use of antibiotics and their correct disposal should be a

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responsibility not only of the health professional but also of the general population. The widespread use of antibiotics has led to an increasing incidence of bacterial resistance, despite the fact that the death rate from MRSA has been decreasing over the years. However, MRSA is the most frequent etiologic agent of antimicrobial-resistant healthcare-associated infections in the world and represents a serious challenge for public health. MRSA has become an obstacle to the treatment of infections and this is mainly due to its innate and acquired virulence factors.

The ineffectiveness of the measures implemented in recent decades to combat the appearance of bacterial strains that are insensitive or not very sensitive to conventional antibiotics indicates that this phenomenon of bacterial resistance is inevitable. However, it is possible to modify the speed with which resistance develops, through various measures such as the rational use of these drugs and the education of patients, aimed at avoiding self-medication and towards exact compliance with the schemes of antibiotics ordered by the doctor. Another important strategy is the adequate selection of the antibiotic regimen, for which it is important to know the class of predominant microbial flora and the prevalence of strains resistant to a certain antibiotic, in the community or in the corresponding health institution. "Bacterial resistance is a constant problem, since the early years of the antibiotic era and one of the factors that generates the most concern is that the interval between the introduction of a new antibiotic and the appearance of resistant strains is increasingly shorter. The selective pressure exerted on the ecosystem of bacteria and the abusive and uncontrolled use of antibiotics allow the selection of germs with more refined and complex resistance mechanisms, so that the appearance of "superbugs" is an increasingly possible possibility near and it is not unreasonable to predict a bleak future, in which antibacterials will lose much of their current usefulness".

This has promoted the investigation of novel alternatives to combat bacteria, including the use of natural defense molecules (cecropins, magamines, melittins, etc.), produced by various species of higher organisms, as well as the design of synthetic canonical peptides. The first promising results indicate that this field is the basis for the development of antimicrobials of the future.

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