Study of the *Enterobacteriaceae* Group CESP (*Citrobacter*, *Enterobacter*, *Serratia*, *Providencia*, *Morganella* and *Hafnia*): A Review

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The CESP group consists of enterobacteria belonging to the genera *Citrobacter, Enterobacter*, *Serratia* and *Providencia*, and more recently, *Morganella* and *Hafnia* genres. Previously known only as commensal micro-organisms are known today to be responsible for serious health problems worldwide mainly in immunocompromised patients, being reported many outbreaks associated with these bacteria. Micro-organisms belonging to the CESP group are related to infections in many sites such as respiratory tract, urinary tract, gastrointestinal, colonizing wounds and devices, especially in hospitalized patients. Are producers of β-lactamase chromosome inducible, such as the AMPc enzyme type that during use of antimicrobials can express an increase in the production of β-lactam resistance. Also noteworthy is the production of β-lactamases of extended spectrum and carbapenemase. It is believed that the transmission of bacteria from CESP group among patients in the hospital environment is amplified due to the lower adherence to hand hygiene by healthcare professionals. Due to its multidrug resistance, its therapeutic option is limited and the best choice is the fourth generation of cephalosporins such as cefepime and tigecycline (glycylcycline).

Keywords: CESP group; multidrug resistance; therapeutic option

1. Introduction

Gram negative bacilli glucose fermenters (*Enterobacteriaceae* family) are involved in almost all infections acquired in the ICU (Intensive Care Unit), particularly respiratory infections and urinary tract infections. The primary agents associated with Gram negative antibiotic resistance is *Enterobacter* spp., *E. coli*, *Klebsiella* spp., *Serratia* spp., *Citrobacter* spp., *Proteus* spp., among others. However, the CESP group has shown high levels of multidrug resistance in clinical strains [1, 2].

The CESP group was previously formed by bacteria of the genus *Citrobacter*, *Enterobacter, Serratia* and *Providencia*, and was recently added to this group the genus *Morganella* and *Hafnia*. Micro-organisms of this group belong to the family *Enterobacteriaceae* and are producers of β-lactamase AmpC (β-lactamases inducible). The β-lactamases AmpC type are enzymes encoded by chromosomal genes and plasmidial [3, 4].

Antibiotic resistance develops as a natural consequence of the ability of the bacterial population to adapt. The indiscriminate use of antibiotics increases the selective pressure and also the opportunity of bacteria be exposed there to, which facilitates the acquisition of resistance mechanisms. Antibiotics act as inducers for the expression of bacterial genes encoding resistance mechanisms to these drugs. The use of these chemicals has revolutionized the approach to infections and their success has generated great optimism about the prevention and treatment of infections. However, the prescription is not always careful and rational of these antimicrobial quickly generated difficulties for its use due to progressive bacterial resistance to these drugs. Antimicrobial resistance has become a major public health problem worldwide, affecting all countries [5, 6, 7].

Thus, a review was conducted of the main aspects related to the enterobacteria and the CESP group, reporting the mechanisms of resistance to antibiotics and informing therapeutic options in order to provide current and relevant information to health professionals consultation.

2. Enterobacteria

The *Enterobacteriaceae* family is the largest and most heterogeneous collection of Gram-negative bacilli of medical importance. The genera belonging to this family were classified based on biochemical properties, antigenic structure, DNA-DNA hybridization and sequencing of 16S rRNA. They are also dispersed in nature and can be found in plants, soil, water, normal microbiota in the intestinal tract of both man and other animals. Microbiological and medical importance stems from the development of infections, as well as pathogenicity and appearance of multi-resistant bacteria to antibiotics used in therapy [2, 8, 9].

Most genus and species belonging to this family have the following properties: they are Gram negative straight, of moderate size (0.3 to 1.0 x 1.0 to 6,0μm), share a common antigen, do not form spores, have motile with 5 polar flagella

in one pole or are without flagella, develop into peptone or meat extract supplement or without added sodium chloride as well as on MacConkey agar. Are grown under aerobic or anaerobic, ferment glucose and other sugars, reduce nitrate to nitrite, is catalase positive, oxidase negative and contains 39% - 59% guanine-cytosine (GC) in their DNA. Most of the micro-organisms have fimbriae, also known as pili, which are subdivided into common fimbriae encoded by chromosomal genes and sex pili which is encoded by genes located on conjugative plasmids. The common fimbriae are important for bacterial adherence to specific receptors on host cells, since the sexual pili facilitates gene transfer between bacteria [2, 9].

Enterobacteria make up about 80% of the strains of medically important Gram negative bacilli and approximately 50% of all bacteria isolated in microbiology laboratories. These micro-organisms are responsible for about half of all cases of sepsis and more than 70% of cases of urinary tract infection, and a considerable percentage of intestinal infections, and may be associated with community and hospital infections. Can also cause infections of surgical wounds, abscesses, pneumonia, sepsis and meningitis [2, 8].

The lipopolysaccharide (LPS) thermostable is the principal antigen of the cell wall and is composed of three components: the somatic polysaccharide O, located more externally, a central polysaccharideto common to all *Enterobacteriaceae* (enterobacter common antigen) and in the internal portion the lipid A . The central polysaccharide is important in the classification of the micro-organism as a member of the family *Enterobacteriaceae*, the polysaccharide O is crucial to the epidemiological classification of strains within species and lipid A, is responsible for endotoxin activity, an important virulence factor [9].

Several members of the family *Enterobacteriaceae* can cause opportunistic infections including septicemia, pneumonia, meningitis and infections of the genito-urinary tract. As an example of genres that can cause these infections are: *Enterobacter, Citrobacter, Escherichia, Providencia, Serratia, Hafnia e Morganella* [10].

3. Group CESP

The CESP group consists of producing enterobacteria of β-lactamases inducible. Belong to this group six genus: *Citrobacter*, *Enterobacter*, *Serratia*, *Providencia*, *Morganella* and *Hafnia*. Are bacteria that have great potential to become resistant during treatment with β-lactam antibiotics. Many of these micro-organisms were classified only as commensals, however, more recently, have been known to be responsible for serious health problems worldwide [11].

3.1 Genus *Citrobacter*

Members of the *Citrobacter* genus are Gram negative bacilli belonging to the family *Enterobacteriaceae*, facultative anaerobic with motility. They may be alone or forming pairs, usually have no capsule and feature motile with 5 polar flagella in one pole. May have respiratory and fermentative metabolism, are oxidase negative, catalase positive, reduce nitrate to nitrite and not decarboxylate lysine [12].

According Koneman et al. (2010) [13] these micro-organisms are able to ferment glucose and produce acid and gas, exhibit positive response for the methyl red test (VM) and negative for the Voges-Proskauer test. Most strains of *C. freundii* are H_2S producers on agar triple sugar (TSI) and are fermenting lactose. However, this fermentation is typically time consuming and does not produce indole.

The *Citrobacter* genus was described in 1932 together with the species *C. freundii*. In 1970 it was described a new species was named *C. koseri* after that a group of similar micro-organisms was called *Levinea malonatica* and in 1972 was described the species *C. diversus*. Years later studies revealed that these three strains were phenotypically similar and it was proposed to *C. koseri* name to sort the three strains analyzed. Currently the *Citrobacter* genus comprises the species *C. farmeri, C. braakii, C. freundii, C. koseri (diversus), C. amalonaticus, C. gilleni, C. murliniae, C. redentium, C. sedlakii, C. werkmanii* and *C. youngae* [13].

Most *Citrobacter* strains are isolated frequently from patients or subjects as a secondary opportunistic pathogen. They make up the normal flora of the human gut, but cause sporadic and epidemic episodes of meningitis, with high incidence of brain abscess and endocarditis in hospitalized patients. Some serotypes of *C. koseri* (*diversus*) can also be enteropathogenic causing diarrhea. They are widely distributed in soil, water, food and the intestinal tract of humans and animals. These bacilli were previously considered purely environmental contaminants or non-pathogenic colonizers but known to be opportunistic pathogens, they can cause serious infections, sepsis, respiratory infections and urinary tract infection, especially in high-risk groups such as infants and immunocompromised adults [14, 15, 16, 17, 18].

Micro-organisms of the genus *Citrobacter* have been linked to a number of diseases, including those of the urinary tract, respiratory tract, wounds, bones, peritoneum endocardium, meninges, intestines and blood stream [19]. Sporadic infections and outbreaks caused by *C. freundii* were reported in 1979 in India, where seventeen babies were infected and one died. Still other cases have been reported such as gastroenteritis by *C. freundii* in nursery school and kindergarten in Germany, which involved 152 cases where these eight patients developed hemolytic uremic syndrome [20, 21]. None et al. (2004) [22] reported a small outbreak of infection caused by the species *C. freundii* resistant to 3rd generation cephalosporin in a surgical center of a university hospital in 2002, which identified four patients with biliary

tract infection and three patients colonized. All cases of infection and colonization were previously patients undergoing surgical procedures.

The species *Citrobacter freundii* isolated from fecal samples have cytotoxic activity, thus being implicated as a cause of gastrointestinal infection. *C. koseri* cause meningitis in human neonates and mastitis in cattle, and can also be a cause of opportunistic infections in other mammals [23]. About 20% of meningoencephalitis caused by Gram negative bacteria cause complications from abscesses. The neonatal meningitis by *Citrobacter koseri* are complicated by abscesses in 40-70% mortality reaches about 30-35% and 90% are caused by the species *C. koseri* followed by *C. freundii* [24].

Several cases of peritonitis caused by *Citrobacter* species have been reported. Plaza (2002) [25] narrated case occurred in Spain, a patient with recurrent episodes of peritonitis in one of the episodes one species of *Citrobacter* were detected as being the causative agent. Nakamoto et al. (2004) [26] reported 99 cases of peritonitis occurring in a nephrology clinic in Japan between 2000 and 2002, of which 3 were caused by *Citrobacter* species and were resistant to treatment. Carlini et al. (2005) [27] reported a case of peritonitis caused by *C. braakii* in an elderly patient in Italy.

Whalen et al. (2007) [28] describe the case of an immunocompromised patient who was admitted to a hospital to perform heart surgery and on the first day after surgery, developed in the fold left inguinal infection by *C. freundii*. Although they are commonly isolated from surgical wounds, urinary tract infections, respiratory and gastrointestinal tracts of immunocompromised patients, *Citrobacter* species can infect any body site in debilitated patients. Reported cases of skin infections *Citrobacter* had the following medical conditions: folliculitis, cellulitis, hives, ulcers and necrotizing fasciitis [29]. Leon et al. (2011) [30] described a *Citrobacter freundii* strain isolated from endoscopic ultrasound fine needle aspiration in a Caucasian male 80 years of age with pancreatic pseudocyst after necrotizing pancreatitis. Acute necrotizing pancreatitis associated with *Citrobacter* infections is rare and so far, few cases have been reported in the literature.

3.2 Genus *Enterobacter*

Among the agents that have been recognized as important pathogens responsible for significant incidence of nosocomial infections, there is the genus *Enterobacter*. This genre is resistant to many antibiotics and quickly develops resistance to new agents. Are opportunistic pathogens that rarely cause primary disease in humans, primarily affecting patients undergoing antibiotic therapy to invasive procedures, particularly in diabetic patients and neutropenic [31].

Enterobacter bacteria are Gram negative, have motility through motile with 5 polar flagella in one pole, ferment glucose with production of acid and gas, does not hydrolyze urea. Serotypes have mostly positive reaction in the Voges-Proskauer test and citrate Simmons, being negative for methyl red and indole test. They also tested positive for ornithine-descaboxilase. The optimum temperature for growth is 30 °C, but most strains grow at 37 °C [32]. The genus consists of 16 species according to their biochemical and genetic characteristics. The species of greatest clinical value are *Enterobacter cloacae* and *Enterobacter aerogenes*, followed by *Enterobacter sakazakii*, which is characterized by the production of a typical yellow pigment. Other species such as *Enterobacter asburiae*, *Enterobacter gergoviae*, *Enterobacter taylorae* (currently *Enterobacter cancerogenus*), *Enterobacter hormaechei*, *Enterobacter amnigenus* and *Enterobacter agglomerans* (removed from the *Enterobacter* genus and renamed as *Pantoea agglomerans*). They are rare in clinical samples [13, 33]. Sources of contamination can be endogenous or exogenous. These bacteria are present in the gastrointestinal tract of man and other animals, water, soil, plants and insects. Outbreaks of infections are common in adult and children's ICU, burn units and cancer centers. Commonly they are associated with contamination by intravenous infusion solutions, blood products, stethoscopes, cotton swabs, tensiometers and colonized hands of health professionals [34].

They are described as agents associated with respiratory infections, skin, urinary tract, bones, joints, central nervous system and gastrointestinal tract. Also cause bacteremia and soft tissue infections, endocarditis, intraabdominal infections, septic arthritis, osteomyelitis, and ophthalmic infections. They have high rates of co-infections with other pathogens, predominantly in the liver and lung infections [16, 33]. Drudy et al. (2006) [35] narrate outbreaks of infections with *Enterobacter sakazakii* in 2004 in a neonatal ICU in New Zealand on this occasion four children developed meningitis and one of them died. *Enterobacter* spp. is mentioned among the four pathogen with the highest incidence in urinary tract infections in hospitalized patients, being among the main microorganisms that develop resistance to antimicrobials in this site of infection. Most of the infections are caused by *E. cloacae, E. aerogenes* and *E. sakasakii* [36, 32].

Enterobacter members of the genus are considered emerging pathogens in hospital infections, ranking third in the family of *Enterobacteriaceae* in the world. In Latin America, this genre ranks fifth among the pathogens in pediatric patients. In Brazil, this pathogen is responsible for 6% to 8.5% of infections in patients in the intensive care unit, being the third most prevalent enterobacteria in urinary tract infections, septicemia and soft tissue [37, 38, 39].

3.3 Genus *Serratia*

The *Serratia* genus consists of aerobic and facultative anaerobic bacteria. They are Gram negative bacilli. The production of hydrolytic enzymes lipase, DNase and gelatinase are features which distinguish the genus *Serratia* from

other members of family *Enterobacteriaceae*. It shows strong positive catalase reaction, negative oxidase, indole variable production; test Voges- Proskauer, Simmons citrate and lysine decarboxylase are negative. They do not produce sulphide and urea is not hydrolysed. Colonies of *Serratia* are mostly opaque, and may be presented iridescent, ie reflect the rainbow colors, white, pink or red. Most colonies grow at temperatures between 10 and 36 °C and at pH 5-9. Because of the virulence of the species *Serratia*, it is essential that their identification is performed correctly separating them of the group *Enterobacter*. The test for detection of extracellular DNase production is reliable, because only the *Serratia* genus produces, making it possible the separation. The differences in the use of carbohydrates such as Ducitol, Adonitol, inositol, sorbitol, arabinose and raffinose, also help in identification. Among the identified species, *Serratia marcescens* is causing the vast majority of human disease. It has been considered a human pathogen since the 1960s includes ten species of bacteria, and in human infections caused by *S. marcescens* is often involved in nosocomial infections. *S. liquefaciens* has been implicated in some human infections and *S. rubidaea* and *S. ficaria* have been isolated from respiratory samples [12, 13, 9].

In hospitals, *S. marcescens* bacteria has been involved in necrotic fasciitis in patients treated with steroids in bullous cellulitis after animal bite and in hemodialysis patients or skin ulcers. The *Serratia* cause opportunistic infections, typically in patients with granulocytopenia and immunosuppressed by disease or therapy. Colonization, surgical complications and trauma may also favor infection. Can cause septicemia, meningitis, endocarditis, and surgical site infections in immunocompromised patients. It is considered a micro-organism difficult to treat because it has a high intrinsic resistance and wide to various antibiotics such as ampicillin, cefazolin and cephalothin [15, 40, 41, 42, 43].

S. marcescens is the most important member of the *Serratia* genus as it is often related to the wide variety of infections, mainly pneumonia and septicemia in patients with reticuloendothelial cancer receiving chemotherapy. Can also cause urinary tract infections and injuries [13]. A study by Lima et al. (2011) [44] reports outbreak of neonatal infection by an endemic clone of *S. marcescens*, which occurred between February and June 2006 in Belém. During this period 35 positive cultures for *S. marcescens* were obtained from blood culture isolates, rectal swab, hand swabs, samples of breath, blood samples collected a few months after the outbreak and 3 isolated from other unspecified locations. As a conclusion, the researchers suggested that cross transmission had occurred among patients during the study period. Infected or colonized newborns were considered the main reservoir in this outbreak.

3.4 Genus *Providencia*

The genus consists of Gram negative members of the *Enterobacteriaceae*. Consist of five species, in decreasing order of prevalence they include *Providencia stuartii*, *P. rettgeri*, *P. alcalifaciens*, *P. rustigianii* and *P. heimbachae*. They are generally considered to be commensals in the gastrointestinal tract, but some species (*P. stuartii* and *P. alcalifaciens*) have been associated with hospital-acquired infections in the elderly and are considered as opportunistic pathogens [13, 45, 46]. The micro-organisms of the genus presents positive catalase reaction, negative oxidase and reduce nitrate to nitrite, do not ferment lactose, present negative reaction to the tests Voges- Proskauer and lysine decarboxylase and positive reaction to the Simmons citrate, for the reaction of methyl red as well as for indole production. They do not produce sulphide and urea is hydrolyzed (only 11-50% of *P. stuarti* strains are positive for the reaction of hydrolysis of urea). All members of this genus desaminam phenylalanine and *P. rettgeri* is the one that is always positive for the hydrolysis of urea [13, 47]. The genus *Providencia* can be found in various animal reservoirs, dogs, cats, cattle, sheep and poultry as well as flies, and often also in soil and sewage water [46, 48, 49, 50].

Providencia stuartii is an opportunistic pathogen involved in community-acquired infections, and in infections acquired in hospitals. *P. stuartii* clinical strains are mostly isolated from urinary tract infections in patients hospitalized for long periods of use of urinary catheters. They may also be isolated, in fewer cases, from respiratory and skin infections. The bacteria *P. stuartii* is reported as more resistant species of the family *Enterobacteriaceae* [50, 51, 52].

Several pathogenies in different animals are related to the genus *Providencia*. In humans, these bacteria cause urinary tract infections, keratitis, dacryocystitis, conjunctivitis and endophthalmitis. Possible risk factors include a compromised ocular surface and co-existing medical morbidity, including urinary tract infections, recent hospitalizations and an immunocompromised state. [53]. *P.rettgeri*, *P. stuartii* and *P. alcalifaciens* has been isolated from samples of human faeces, either as part of the human intestinal microbiota as well as a cause of gastric disorder, such as traveler's diarrhea [54]. In 2001, *P. alcalifaciens* was implicated in a major outbreak of foodborne illness among children in Japan. The species *P. rettgeri* has affected a range of hosts and have been isolated from poultry, reptiles and amphibians [55] .The emergence of resistance in *Providencia* spp. is a clinical concern as it may lead to increased treatment costs and delays in treatment, which can be fatal to the patient. Strains of *Providencia* spp. resistant to antibiotics may also play an important role in spreading resistance through exchange with antibiotic resistance genes with other pathogenic bacteria [56].

3.5 Genus *Morganella*

Micro-organisms belonging to the genus *Morganella* show positive catalase reactions, oxidase negative and reduce nitrate to nitrite. Non- fermenting lactose, present negative reaction to the Voges- Proskauer tests, variable response to lysine decarboxylase and negative for the Simmons citrate. Positive reaction for the Methyl Red reaction, as well as for

indole production. Most strains do not produce sulphide and urea is hydrolyzed [47]. The flagella of species *Morganella* and its membership are the main determinants of colonization and formation of bacterial biofilms. Several fimbriae demonstrated important role in establishing urinary tract infections. The urease activity is a factor that affects the growth of bacteria and biofilm formation in the urinary tract. Despite its wide distribution, gender is a rare cause of infection acquired and is most often found in postoperative infection, causing urinary tract infection, septicemia, pneumonia, central nervous system infections, meningitis and other nosocomial infections, may cause until death [57, 58, 59].

Morganella morganii Gram negative bacteria that presents a negative result for the test Simmons citrate, as well as hydrogen sulfide, with variables for the test of ornithine decarboxylase. Although it was previously classified as *Proteus morganii*, this was attributed to the genus *Morganella* based on results of DNA-DNA hybridization. These studies made it possible to divide this genus into three groups and seven subgroups. Members of this genus can be fermenters trehalose, expressing decarboxylase of lysine and ornithine. *M. morganii* that not ferment trehalose are classified as of *M. morganii* subspecies *morganii* and those capable to ferment it as subspecies *sibonii* [13, 60, 61].

Morganella morganii has been implicated as a cause of diarrhea, urinary tract infections and wounds. Serious infections like meningitis in patients with AIDS and brain abscess in nursing have been reported in the literature. Other individual cases of infection and nosocomial outbreaks have shown that infection may lead to serious medical problems that are generally associated with common causes of bacteriuria associated with use of a catheter, wound infection, urinary tract infections, hepatobiliary tract and septicemia [13, 62, 63]. Clinically significant infections include sepsis, chorioamnionitis, urinary tract infections, hepatobiliary tract and surgical wounds. Occasional invasive infections such as pericarditis, septic arthritis and endophthalmitis were reported in patients undergoing recent invasive procedures with chronic morbidity or subjected to prolonged hospitalization [63, 64, 65]. Tsai et al. (2013) [66] report evidence suggesting that *M. morganii* species may become an important opportunistic pathogen in urinary tract, skin and soft tissue infections and also in bacteremia. The authors describe the case of a woman in continuous ambulatory peritoneal dialysis (CAPD), which presented the first case of CAPD related peritonitis in a monobacterian environment. The *Morganella* genus have been reported as part of the normal microbiota of ophidians, both in the oral cavity as in cloacal. According to the Ministry of Health *M. morganii* is one of the bacterial agents involved in local complications by secondary necrosis in humans after the accident ophidian [67].

3.6 Genus *Hafnia*

Hafnia alvei is the only species of the genus *Hafnia* described. Was previously classified as *Enterobacter hafnia* due to their biochemical characteristics resemble the species of *Enterobacter*. What differs is the fact that *H. alvei* not produce acid from lactose, sucrose, melibiose, raffinose, adonitol, sorbitol, dulcitol and inositol. Another important feature is that *Hafnia* does not produce lipase or desorribonuclease, which differ from the *Serratia* genus and also have a feature to exhale strong odor of human feces [13]. The species is catalase positive, oxidase negative and reducing nitrate to nitrite. Do not ferment lactose, presents positive reaction to the tests Voges- Proskauer and lysine decarboxylase and the Simmons citrate. Negative reaction in methyl red test, as well as for indole production. Do not produce sulfide and urea is not hydrolysed [12]. In the pathogenesis participates lipopolysaccharide (LPS) corresponding to the antigen O [15, 68].

H. alvei is normally considered a colonizing organism and rarely appears as a pathogen. Pulmonary infection is rare and appears associated with prolonged mechanical ventilation and immunosuppression of the host and also an ability to generate rapid resistance [15]. Commonly shows susceptibility to quinolones, aminoglycosides, chloramphenicol, cotrimoxazole, cefepime, aztreonam and carbapenem, while susceptibility to tetracycline is variable [69].

According Koneman et al. (2010) [13] the clinical relevance of *H. alvei* is not clear, since it has been isolated from human feces in asymptomatic patients. However, isolated cases have been described of infections in wounds, abscesses, sputum, urine samples, blood and other locations. In the cases described in the literature, isolation is associated with polymicrobial infection, which hinders their role as causative agent. There have been reported cases of gastroenteritis, necrotizing enterocolitis, cholecystitis pyogenic, peritonitis, meningitis, urinary tract infections, skin abscess, endophthalmitis, bacteremia, endocarditis, pneumonia, empyema and wound infections in hospitalized patients. They can be isolated from samples of orofaringes, gastrointestinal, and less frequently in blood cultures, urine, tissues, and cultivation of endovascular catheters [15, 70]. Nosocomial infections include cholecystitis, infections in the respiratory, hepatic and pancreatic pathways have also been associated with sporadic enteritis, conjunctivitis, conjoint infections, wounds pleuritis, peritonitis and bacteremia [15, 71, 72, 73]. There are also reports of several people who have suffered bacteremia by *Hafnia* after liver transplant. Another case occurred with patient wounded by bullet and underwent to surgery. Two cases of pneumatosis intestinalis accompanied by bacteremia by *H.alvei* have also been described, one in a boy 6 years old with leukemia and another in a child 20 days of age with necrotizing enterocolitis [74, 75, 76, 77].

4. Multidrug resistance of CESP group to antimicrobial

The impact of bacterial resistance is widespread and constitutes a serious threat to humanity. It is considered a public health problem, which includes the medical and social areas. If these bacteria are not inhibited in the future they will be even more devastating for humanity, compared to what was experienced in the era pre-antibiotic, since the emergence of new therapeutic resources does not follow the evolution of resistance mechanisms [78, 79, 80, 81]. The ability to multiply rapidly, being vulnerable, mutate and the possibility to exchange genetic material both from the same species as strains from different species, make the bacteria, micro-organisms with high adaptability to various factors, such as, exposure to potent chemicals [82]. Despite the antimicrobial resistance to be predominantly related in hospital system, resistant bacteria can also be found spread in communities. The spread of bacterial resistance to antimicrobial agents is a result of natural selection pressure. This pressure can result in the growth of pre-sensitive strains that acquire resistance (acquired resistance) or emergency strains which are normally resistant (intrinsic resistance) [16].

Although the mechanisms of resistance may vary from pathogen to pathogen is caused by some basic factors such as: inactivation of antibiotic directly to the bioactive molecule by chemical modification, usually promoted by bacterial enzymes; target modification that leads to loss of sensitivity to the antibiotic; changes in efflux pump and outer membrane permeability to promote the reduction of the concentration of the antibiotic without chemical modification and the target transmission [83, 84, 85].

The multidrug resistance phenomenon is present in all bacterial species. The multi-drug resistance mechanisms typically include a reduction of porins synthesis and increased efflux pumps in order to decrease the concentration inside the bacterial cell different antimicrobials, including, β-lactams, tetracyclines, chloramphenicol, aminoglycosides and quinolones. The resistant phenotype is the result of chromosomal gene activation by induction or mutation and DNA transfer. The efflux mechanism is characterized by the expulsion of drug from the interior of the bacteria as a consequence of overexpression of efflux pump systems. This efflux mechanism contributes to the intrinsic and acquired resistance in enterobacteria [86, 87]. Gram negative bacteria are generally more resistant to a large number of antimicrobial agents that Gram positive. It is believed to be due to their structural characteristics, with the outer membrane functioning as a barrier to access of drug to its intracellular target sites [16].

According to Vermelho (2007) [16], the majority of antimicrobial drugs in use is derived metabolites of soil organisms, particularly fungi and actinomycetes. These antibiotic-producing organisms also exhibit the same resistance mechanisms that have been identified in pathogenic bacteria. Therefore, it is believed that the resistance genes evolved in organisms producers of antibiotics agencies to protect them from inhibitory action of its own antibiotic. Events subsequent of horizontal transmission may have widespread resistance determinants for these other micro-organisms.Strains belonging to the group of bacteria known as CESP are constitutive producers of chromosomal β-lactamases such as the type AmpC enzymes that during the use of antimicrobials can express an increase in the production of the β-lactam resistance. AmpC-producing strains may eventually express sensitivity in susceptibility testing, while mutant strains express resistance. The expression of sensitivity or resistance, "*in vitro*" depends on the enzyme concentration [88].

The spread of antibiotic resistance genes can occur by two mechanisms, vertical transmission, by cell division generating new cell identical, or by horizontal transmission when occurs exchange of genetic material between bacteria of the same species or different species, by methods such as: conjugation, transduction, transformation and implementation. The horizontal transfer of resistance genes and the co-existence of genes which confer resistance to several antibiotics in the same movable member, enables the survival of bacteria under selective pressure of different classes of antibiotics [81, 86, 89, 90].

Klebsiella pneumoniae carbapenemase (KPC) is an important resistance mechanism in the global hospital setting. The KPC is an enzyme produced by Gram negative bacteria (enterobacteria) and their detection in bacterial isolates confer resistance to carbapenem antibiotics, and inactivate penicillins, cephalosporins and monobactams. Importantly, carbapenems comprise a class widely used in the treatment of infections involving multiresistant enterobacteria [91, 92, 93]. KPC enzyme was detected in different bacteria through molecular studies and classified into KPC-1, KPC 4, with the following description: The enzyme KPC-1 was initially described as a strain of *Klebsiella pneumoniae*; KPC-2 in *K. pneumoniae*, *K. oxytoca*, *Salmonella enterica* and *Enterobacter* spp.; KPC-3 in *K. pneumoniae* and *Enterobacter cloacae*. For KPC-4, they were not found related microorganisms. Bacteria that produce KPC enzymes contain the *bla* gene encoding the production of carbapenemases which is an enzyme mediated by plasmid. Other species can produce KPC as *Citrobacter freundii*, *Enterobacter* spp., *Escherchia coli*, *Salmonella* spp., *Serratia* spp. and *Pseudomonas aeruginosa* [94, 95, 96, 97].

A study in 2001 found that *Enterobacter* spp. showed high rates of resistance to third-generation cephalosporins, monobactams and broad-spectrum penicillins associated with β-lactamase inhibitors. About 40% of the samples tested were resistant to ceftazidime, ceftriaxone, aztreonam and piperacillin / tazobactam [37]. Increasing antimicrobial resistance among bacteria of the CESP group has caused many limitations on treatment options. Missed detections of different resistance phenotypes may cause inappropriate use of antimicrobials, disadvantaging clinical outcomes and causing uncontrolled spread of resistant bacteria [98].

4.1 Resistance to β-lactam

The β-lactam antibiotics are an example of action at the level of synthesis of peptidoglycan present in the bacterial cell wall, they are quite prescribed because of their low toxicity and therapeutic efficacy. This group includes penicillins, cephalosporins, carbapenems, monobactams and some inhibitors of β-lactamase. All β-lactam contain in their molecular structure an β-lactam ring, differing in side chains [99]. The resistance of Gram negative bacteria to β-lactam antibiotics is due to the production of β-lactamases are enzymes, that catalyze the hydrolysis of the β-lactam ring, preventing thus their antimicrobial activity. The production of β-lactamases extended spectrum (ESBL) by Gram negative bacteria make these micro-organisms resistant to most β-lactams, including the penicillins, cephalosporins and spread spectrum monobactams such as aztreonam. Members of the family *Enterobacteriaceae* commonly expressed β-lactamase encoded by genes located in chromosomes or plasmids, capable of replication and spread among bacteria of different species and between different genus [3, 100, 101, 102]. *Citrobacter freundii* has natural resistance to ampicillin, amoxicillin, amoxicillin + clavulanic acid, 1st generation of cephalosporins, cefoxitin, cefuroxime and cefotetan. *C. diversus* already is resistant to ampicillin, amoxicillin, carbenicillin and ticarcillin. The *Enterobacter* genus is naturally resistant to ampicillin, amoxicillin, amoxicillin + clavulanic acid, 1st generation of cephalosporins, cefoxitin, cefuroxime and cefotetan. The natural resistance of the genus *Providencia* covers drugs: ampicillin, amoxicillin, amoxicillin + clavulanic acid, 1st generation of cephalosporins, netilmicin, tobramycin, nitrofurantoin, cefuroxime, gentamicin, polymyxin and colistin. Genus *Serratia* is resistant to ampicillin, amoxicillin, amoxicillin + clavulanic acid, cefuroxime, colistin, 1st generation of cephalosporins and polymyxins. In addition to these antibiotics *Serratia marcescens* is also resistant to cefoxitin and nitrofurantoin [103]. *Serratia marcescens* are reported in many hospitals, have high rates of resistance to quinolones, β-lactams and aminoglycosides in general, for the production of β-lactamase [2]. Many strains of M. *morganii* are resistant to drugs cefazolin, cefixime, cefpodoxime and ampicillin. Drug resistance was introduced through additional genetic elements and / or mobile elements. Resistant strains that carry blaCTX-M gene are capable to produce β-lactamases that can break the β-lactam drugs extended spectrum [104, 105, 106].

β-lactamase enzymes represent acquired bacterial resistance which genes are typically contained on the plasmids, which gives the spread of genetic information, future generations of the same species or of different species [107, 108]. The β-lactamase may operate by two different mechanisms of action, by zinc ions which are capable of breaking the β-lactam ring in the case of metallo-β-lactamase, or the use of ester-serine via, in the case of other β-lactamases of classes [86].

Bacteria that possess gene cAMP chromosomal produces β- lactamase cAMP in a basal level, having physiological function in cell wall metabolism. However, this feature confers decreased sensitivity or resistance to cephamycins, such as cefoxitin. This intrinsic characteristic of resistance also gives decreased sensitivity or resistance to penicillins, cephalosporins of 1st and 2nd generation and antibiotics associations with β-lactamase inhibitors. In bacteria of CESP group resistance to 3rd generation cephalosporin may occur when there is overproduction of β-lactamases AmpC [86, 109]. The enzymes AmpC type are chromosomal and have their expression inducible, being produced only in insignificant quantities in the absence of β-lactam antibiotics and in large quantities when they are present. The class B or metallo-β-lactamases are plasmidial and require zinc for their action, and are capable of hydrolyzing β-lactams of all chemical classes, excluding monobactams and are not inhibited by β- lactamase inhibitors such as clavulanate or tazobactam [110, 111]. The isolated of CESP group when exposed to an inducer agent, usually a β-lactam, begin to produce high amounts of cAMP. It is the permanent expression of the high level of enzyme, known as derepressed state, which confers resistance to third-generation cephalosporins, when produced by mutations in regulatory genes. These mutants can be selected during treatment with certain β-lactam antibiotics [112, 113].

The production of β-lactamases extended spectrum (Extended-Spectrum Beta-lactamase ESBL) mainly in some species of Gram negative bacteria is recognized as a problem for hospitalized patients. In recent years, the frequency of ESBL greatly increased worldwide and is a major therapeutic problem in many institutions [114, 115]. ESBLs are enzymes that promote resistance to broad spectrum cephalosporins, penicillins and monobactams, while the sensitivity to cephamycins (cefoxitin) and carbapenems is preserved. The prevalence of ESBL production among Gram-negative bacilli are varied in different countries and also within each geographical location, possibly due to the use of larger, smaller or even indiscriminate, certain antimicrobials. These enzymes are usually caused by mutation in the TEM-1 and SHV-1 genes in plasmids and are easily transmitted from one organism to another [116] . Although they are most frequently identified in *Klebsiella* spp strains, the ESBL may be produced by micro-organisms of CESP group as *Citrobacter diversus*, *C. freundii*, *Enterobacter cloacae*, *E. aerogenes*, *Serratia marcescens*, and reported as a problem in therapy of infections by these bacteria [88].

The carbapenems are a type of antimicrobial therapy of choice for treatment of severe hospital infections caused by Gram negative bacteria (enterobacteria) multidrug-resistant due to the broad spectrum of bactericidal action and its stability against most of β-lactamases, including extended-spectrum - ESBL [93, 117]. Increased rates of Gram negative bacilli resistant to carbapenems by producing β-lactamases that hydrolyze this group of antimicrobial agents has created great concern. The carbapenases are formed from a heterogeneous combination of β-lactamases of class A (penicillinases), B (metalloenzymes) and D (oxacilinases). These enzymes are able to hydrolyze carbapenems and in addition, can also hydrolyze penicillins, cephalosporins and monobactams [118, 119].Carbapenemases of A class are β-lactamases wich suffer inhibition by clavulanic acid, have the ability to hydrolyze β-lactam antibiotics, including

carbapenems, have been reported in lineage of *Enterobacter* spp. with reduced susceptibility to imipenem. The carbapenemases (as NMC-A and KPC-1) can be found in *Enterobacter cloacae* and *Serratia marcescens* [120, 121]. The carbapenemases belonging to class B hydrolyze all β-lactams, excetos the monobactams (aztreonan) and show resistance to β-lactamases inhibitors. These enzymes is described in different pathogens such as *Pseudomonas* spp., *Acinetobacter* spp. and members of the family *Enterobacteriaceae* [104, 118].

The synthesis of β- lactamase enzymes is the major mechanism of resistance to β- lactams, however there are other mechanisms to promote this resistance, such as changes of the target of the antibiotic, the PBPs (Penicillin Binding Proteins), impermeability of the membrane cytoplasmic and presence of efflux protein [102]. The PBPs appears by amino acid substitutions in protein which is the target of the antibiotic. The mechanism of inactivation of antibiotics is based on the transfer groups in which stand the transferase enzymes. Thus antibiotic-target binding is compromised [81, 122, 123]. The efflux pumps are proteins present in the membranes. In this type of resistance occurs in the active transport of antibiotics from intracellular to the extracellular medium. The efflux of the antimicrobial agent can confer a residual level of resistance. This mechanism might not be enough to express clinical resistance, but in conjunction with other mechanisms may be at the origin of treatment failures. The specificity of the antibiotic may vary depending on the efflux pump. The resistance is often caused by increased of the synthesis of the proteins which as a whole constitute the efflux pump which may have affinity for different antibiotics and are often associated with phenomena of multidrug resistance [122, 124].

Generally gram-negative bacteria are intrinsically resistant to glycopeptides vancomycin and teicoplanin. This occurs at high molecular weight of these molecules, preventing their diffusion through the channels of porins present in the outer membrane [107]. Antibiotic resistance is also often associated with a decrease in permeability that occurs in the outer membrane of Gram negative bacteria. The flux of molecules into the cell is ensured by complex of membrane proteins, called OMPs (Outer Membrane Proteins), which form channels. The passage of molecules into the cell is influenced by its load, structure and size. Antibiotics are some of the molecules which use this route to reach the inside of the bacterial cell or, in the case of β-lactam antibiotics, the periplasmic space [86].

4.2 Therapeutic options

Enterobacteriaceae producers of β-lactamase chromosome inducible, CESP group, are bacteria with potential to become resistant during treatment with β-lactam. The best choice are the fourth generation of cephalosporins such as cefepime. Also can be used fluoroquinolones if the infection site is urinary and carbapenems when agents are proven sensitive to these antibiotics. The ESBL-producing micro-organisms are resistant to third-generation cephalosporins and sensitivity to the second generation. Carbapenems are presented as the choice most appropriate therapy [11]. Another treatment option is the use of tigecycline antibiotic which originated from the tetracycline belonging to a new class the glycylcycline, which acts as tetracycline inhibiting protein synthesis at the 30S subunit. Tigecycline retains activity against Gram negative of β- lactamase producers and resistant to all of the β- lactam antibiotics, however it was evidenced that for *Pseudomonas aeruginosa*, *Proteus* spp., *Morganella morganii* and species of *Providencia* the activity was limited [125, 126].

5. Conclusion

Bacteria belonging to the CESP group were not previously considered as pathological agents, only commensals, but increased concern due to the increased number of infections, especially nosocomial associated with this group of bacteria as well as the emergence of multi-resistant micro-organisms to drugs commonly used in the treatment. Antibiotic resistance by enterobacteria of the CESP group is a serious public health problem, and the knowledge of bacterial resistance mechanisms is very relevant, because it allows limiting its spread, helping to reduce the morbidity and mortality rates that are associated with infection by multi-resistant bacteria. The correct use of antibiotics is essential because its inadequate use and non compliance with their prescription are directly related to acquisition of resistance. For the treatment of bacterial infections group CESP drugs of choice are the fourth-generation cephalosporins, tigecycline plus flouroquinolonas and carbapenems.

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