KLEBSIELLA PNEUMONIAE – CAUSATIVE AGENT OF ENTEROCOLITIS. A BRIEF LITERATURE REVIEW.

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ABSTRACT

Gastrointestinal diseases have one of the highest incidence rates worldwide. *Klebsiella spp*., which is a part of the large family *Enterobacterales*, isolated from fecal samples is considered as a part of the normal intestinal flora, even when it presents as a monoculture. The transmission of virulent plasmids from *E. coli* strains to *Klebsiella spp*, raises the question whether those bacteria can be an etiological factor for severe diarrhoea. By using PCR methods, the *lth* gene which is coding heat-labile enterotoxin (LT) was presented in the plasmids of *Klebsiella spp* strains, and its expression was assessed.by measuring the cytopathic effect induced by the LT toxin,

For the first time in 1882, Carl Friedlander isolated *K. pneumoniae* from lung samples from patients dead of pneumonia [1]. *K. pneumoniae* species includes closely related to *K. pneumoniae Kp1-Kp7*. These phylogroups include the following subspecies of *K. pneumoniae subsp. ozaenae, K. pneumoniae subsp. pneumoniae, K. pneumoniae subsp. rhinoscleromatis, K. quasipneumoniae subsp. quasipneumoniae, K. quasipneumoniae subsp. similipneumoniae, K. variicola subsp. variicola, K. variicola subsp. tropica, K. africana and K. quasivariicola* [2,3]. Based on its capsular antigens, *Klebsiella pneumoniae* can be distinguished and classified by serotyping. Currently, 77 serotypes belong to the K-antigen and another 12 belong to the O-antigen serotype. *K. pneumoniae* can be found in nature, as well as in animals, plants, and humans. [4].

K. pneumoniae colonizes human nasopharynx and gastrointestinal tract. The bacterium is a frequent causative agent of urological tract infections (UTIs), meningitis, sepsis, and wound infections. It is for this reason that for many years the isolation of *Klebsiella spp*. from faecal samples has not been a concern to specialists and is regarded as a part of the normal intestinal flora. Even when isolated from diarrhoeal stools in monoculture, these microorganisms have not been treated as an etiological agent of gastrointestinal infections. In 1975, were obtained the first data on the ability of *Klebsiella pneumoniae* strains isolated from tropical sprue patients to secrete enterotoxin [5]. Enterotoxin activity has been shown to induce secretion of water and electrolytes and to cause structural changes in the intestinal mucosa in various animals [6]. In 1976, thermostable (ST) toxin and thermolabile (LT) toxin were discovered [7]. Later in 1983, Klipstein and Engert demonstrated that partially purified filtrates of LT toxin-producing strains were able to stimulate water and electrolyte secretion in the rat intestine. Also, oral administration of LT toxin-producing strains to piglets resulted in severe diarrhoea [8].

Colonization of gastroinsestinal tract of healthy people with unspecified pathotypes ranges from 5 to 35% in Western countries [9, 10], whereas in Asian countries the percentage is higher: from 19 to 88% [11]. During warm months, the incidence of infections with *K. pneumoniae* via the blood route is 1.5-fold higher, reflecting the increased incidence of faecal carriage in humans during summer [12]. Screening of healthy individuals is recommended for the detection of new resistant and virulent strains as well as to gain insight into strain diversity. [13].

The pathogenetic changes caused by *K. pneumoniae* in the gastrointestinal tract are no less complicated than that in urinary tract infections, meningitis, etc. To promote its growth and survival in the host, the bacterium excretes and chelates iron from the host's haemoglobin and transferrin by secretion of siderophores [14]. The host fights bacterial infection

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by secreting free iron found in plasma and binding it to an innate protective protein called lactoferrin [15]. The reduction of free iron in the host environment causes bacteria to secrete siderophores that bind competitively with iron due to its high affinity, chelating it from host proteins such as haemoglobin, ferritin and myoglobin [16]. The ability of a bacterium to secrete more than one type of siderophore increases its ability to colonize the gastrointestinal tract and prevent the neutralization of a single siderophore by the host. The most abundant siderophore secreted by *K. pneumoniae* is enterobactin due to its high affinity for iron [17]. Another factor of *K. pneumoniae* virulence is lipopolysaccharide (LPS), an endotoxin that protects bacteria from host humoral defences [18]. This is due to the ability of *K. pneumoniae* to modify the structure of its LPS, in particular the lipid A moiety, in a way that it would not activate host inflammatory responses, thus increasing bacterial virulence.

During the colonization of the gastrointestinal tract, *K. pneumoniae* causes damage to the intestinal mucosa by secreting a potent toxin called colibactin, which is also produced by some other members of the *Enterobacterales* order, including *E. coli*. The colibactin toxin induces genomic instability by disrupting the cell cycle and DNA repair machinery [19]. Colibactin also activates the senescence-associated secreted protein (SASP) phenotype that promotes tumour development [20]. Once the cell ages, it is inert to mitogenic and oncogenic stimuli, which would be beneficial. In the last decade, however, new evidence has revealed that SASP can be a double-edged sword. During cell senescence, there is increased secretion of inflammatory cytokines, chemokines, growth factors and matrix metalloproteinases (MMPs) into the surrounding tissue microenvironment. This can lead to the development of diseases associated with gastrointestinal inflammation.

K. pneumoniae is classified as an extracellular bacterium, however, in vivo and in vitro studies have also demonstrated the capacity of *K. pneumoniae* to invade and persist in intestinal epithelial cells [21]. Intracellularly, the bacterium can grow, replicate and exit the basement membrane. The potential of *K. pneumoniae* to invade and develop intracellularly, coupled with the release of colibactin toxin, can exacerbate inflammation and disease development. Prolonged exposure to virulent strains of *K. pneumoniae* and their colonization in the gastrointestinal tract can lead to the development of inflammation-induced diseases such as Crohn's disease, ulcerative colitis, and irritable bowel disease. Studies have documented an increased prevalence of *K. pneumoniae* in patients suffering from ulcerative colitis compared to healthy controls. One study revealed that increased colonization of *K. pneumoniae* in the colon would lead to colitis. *K. pneumoniae* can increase the expression of cyclooxygenase (COX)-2, IL-6, IL-1β, and TNF-α, which are potent proinflammatory stimulators [22]. They also increase the levels of lipid peroxidation in the colon [23, 24]. *K. pneumoniae* also increases the production of β-glucuronidase and LPS, which induce NO and COX-2 production in murine peritoneal macrophages and as a result suppresses host immune responses. The protective role of lactic acid bacteria such as Lactobacillus sp. and others was suggested to reduce inflammation caused by pathogenic intestinal bacteria. Thus, dysbiosis of gut microbiota, with increased population of *K. pneumonia, and* reduced population of protective lactic acid bacteria, is associated with inflammatory diseases such as Crohn's disease and ulcerative colitis [25]. Crohn's disease is an idiopathic form of inflammatory bowel disease affecting the terminal ileum that develops following chronic generalized enteritis. Affecting mainly individuals from 20 to 30 years, it has a higher prevalence in developed countries [26,27]. The pathogenesis mechanism of Crohn's disease is charcterized with recurrent subclinical infections leading to increased *K. Pneumoniae* specific antibody titers cross-reacting and binding to intestinal collagen fibres of the terminal ileum, and activating the complement pathway and proinflammatory cascades. The influx of proinflammatory cytokines into the terminal ileum induces inflammation. Recurrent infections with *K. pneumoniae* lead to a continuous cycle of damage to the colonic mucosa by various cytokines, ultimately leading to the development of Crohn's disease.

In the last decade, the role of *Klebsiella spp*. produced enterotoxins (LT) as virulence factors in the pathogenesis of diarrhoea has been revealed. These toxins are thermolabile and cytotoxic to the intestinal

epithelium, similar to the interpolable toxins produced by enterotoxigenic *E. coli (ETE*C) strains, which are an etiological factor for severe dehydrating diarrhoea in humans and animals [28,29]. The genes encoding enterotoxins are predominantly located on plasmids [30] and can therefore be transferred between gram-negative microorganisms such as *Klebsiella pneumoniae, Citrobacter freundii, Yersinia enterocolitica, Enterobacter cloacae and Aeromonas hydrophila* [31,32,33]. The production of LT toxins depends on external factors such as temperature, pH, osmotic stress and concentration of nutrients in the medium. The cytopathic effect induced by *K. pneumoniae* is weaker compared to the effect induced by enterotoxigenic strains of E. coli. Studies have shown that the gene for enteric labile toxin lth is present in *Klebsiella pneumoniae and Klebsiella oxytoca* strains isolated from patients with diarrhoea [34]. Persistent toxin infectious- and haemo-colitis syndromes are demonstrated in patients with concomitant diseases and infancy. Gastrointestinal infection by *Klebsiella pneumoniae and Klebsiella oxytoca* may proceed with the clinic of viral enteric infection. Infections caused by *K. ohutosa* present with severe toxaemia and haemocolitis in patients with concomitant diseases and infancy. However, Klebsiella bacterial enteric infection does not always proceed with hemorrhagic diarrhoea [35]. *Klebsiella spp*. enterotoxins can be important pathogenic factors causing diarrhoea in humans, which turns them from opportunistic to pathogenic bacteria, especially in the immunocompromised, and in patients with gut dysbiosis following antibiotic therapy.

In conclusion, the prophylaxis and early diagnosis of Klebsiella spp. with evidence of toxin production, especially among hospitalized patients, is of utmost importance to prevent the dissemination of antimicrobial resistance, which has been intensively increasing among the genus.

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