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Expert Information

From the Working Group on Hygiene

Implication of infectious agents on results of animal experiments

Klebsiella spp.

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Contents

Klebsiella spp.

Background

- The genus *Klebsiella*, family *Enterobacteriaceae*, was named by V. Trevisan in 1885 in honor of the German bacteriologist Theodor Albrecht Edwin Klebs.[1]
- The opportunistic pathogens *Klebsiella (K.)* spp. are reported in laboratory rodents mainly as *K. oxytoca* and *K. pneumoniae*.[2]
- Ubiquitous in nature; can be frequently isolated from surface water, sewage, soil, and plants[3] and are able to form biofilms.[4,5]

Prevalence

- Worldwide distribution in laboratory mice[6,7]
- *K. oxytoca* was detected by culture in 0.38% and *K. pneumoniae* in 0.1% of mouse samples from facilities in North America and Europe.[7]
- The prevalence in rat samples was 0.37% for *K. oxytoca* and 0.53% for *K. pneumoniae*.[7]

Host species

- All known mammalian species, including common laboratory rodent and lagomorph species, as well as many other vertebrates and invertebrates, are susceptible to colonization with *Klebsiella* spp.[8]
- Frequently isolated from faeces of domestic and wild animal species and from humans[3,9]

Properties

- Gram-negative, non-motile, facultative anaerobic rod-shaped bacteria, usually encapsulated, 0.3-1 µm in diameter by 0.6-6 µm in length[3]
- *Klebsiella* spp. have no complex nutritional requirements and grow on nutrient agar medium (NAM), MacConkey, or blood agar plates.
- Virulence factors may include the polysaccharide-containing capsule, endotoxins, adhesion antigens, and enterotoxins.[9]
- *K. oxytoca* enterotoxins tilivalline and tilimycin are related to antibiotic-associated haemorrhagic colitis in human.[10]
- Cytotoxin tilivalline production appears to be regulated by the environment with soy-based product found to have a strong toxin induction property.[11]
- Environmental *K. pneumoniae* strains were found to be as virulent as strains of clinical origin evaluated in an animal model based on urinary tract infection and intestinal colonization.[12,13]
- Transmission is primarily faecal-oral; aerosol transmission is also effective.[14]

Susceptibility

- Mouse strains C3H/HeJ and C57BL/10ScCr that are lipopolysaccharide hypo-responsive due to toll-like receptor 4 defects were shown to be highly susceptible to infections of gram-negative bacteria.[15-17]

- Infections were reported in immunodeficient NMRI-*Foxn1*^{nu} mice lacking a thymus.[16]
- It has been discussed that an increased susceptibility to keratoconjunctivitis might be caused by a lack of functioning eyelashes due to severe follicular dystrophy.[18]
- Host susceptibility to *K. pneumoniae* is controlled by multiple genetic factors that act sequentially during the course of infection.[19]

Organotropism

- Medically relevant *Klebsiella* spp. colonize the gut preferentially.[20,21]
- Following haematogenous spread, focal abscess formation can occur in any organ.[20]

Clinical disease

- Reports of clinical disease in immunocompetent rodents are rare.[22,23] Clinical signs in immunocompromised rodents are generally more severe.[20]
- There is no pattern of infection or characteristic *Klebsiella*-associated lesion. Clinical signs are those generally associated with Gram-negative bacterial infections, such as poor body condition, ruffled hair coat, otitis media, urogenital tract infections, abscesses, or sepsis.[8]
- Diseased mice show non-specific signs of dyspnea, sneezing, cervical lymphadenopathy, inappetence, hunched posture, and rough hair coat.[22,24]
- Diseased Wistar rats showed unilateral and bilateral fluctuating masses in the cervical and inguinal areas, and focal cutaneous ulcers in the ventral neck.[23]
- Infected NSG mice showed hunched postures and scruffy hair coats, others appeared moribund in lateral recumbency.[25]
- *Klebsiella* spp. frequently cause human nosocomial infections. Especially *K. pneumoniae* is associated with severe pneumonias in humans and also involved in urinary tract infections, otitis media, meningitis, wound infections, abscesses and septicaemia.[9,26-28]

Pathology

- Mice with natural *K. pneumoniae* infection showed cervical lymphadenitis, abscesses (cervical, pharyngeal, renal and hepatic), empyema and granulomatous pneumonia.[22,25]
- Rats with natural disease showed abscesses (lymph nodes, renal and salivary glands) as well as small white foci on their spleens.[23]
- Suppurative endometritis, salpingitis, perioophoritis and peritonitis were identified in aging B6C3F1 mice using cultural isolations from lesions most likely caused by *K. oxytoca*.[29,30]
- Gross necropsy and histology of 90 mice (C3H/HeJ, C3H/HeJZtm and NMRI-Foxn1^{nu}) and rats (LEW.1AR-iddm) infected by *K. oxytoca* resulted in about 80% of the following lesions: otitis media, urogenital tract infections and pneumoniae. Besides this, subcutaneous, intra-abdominal and liver abscesses, keratoconjunctivitis, Harderian gland adenitis, meningitis, infections of the oral cavity, maxilla and salivary glands were reported. Lesions showed severe suppuration and extensive necrosis. Processes were reported to spread to neighbouring tissue.[16]
- Gross necropsy of infected NSG mice revealed mottling of the kidneys and acute or chronic renal inflammatory lesions. These were characterized by bacterial colonies

scattered throughout the renal interstitium and within the distal and proximal tubules and rarely within tubular epithelial cells (acute lesions) or multifocal interstitial fibrosis with tubular degeneration and loss (chronic lesions). Some mice showed ascending urinary tract infection and bacteraemia.[25]

Morbidity and mortality

- Experimental infections of diabetic mice with more virulent strains of *K. pneumoniae* lead to high blood bacterial counts and appearance of bacteria in the liver sinus and cells, which was usually related to death of the animals.[31]
- Nephritis caused by *Enterococcus* sp. or *K. oxytoca* was demonstrated to be a major contributor to morbidity in NSG mice.[25]

Zoonotic potential

- Due to its ability to colonize a wide range of species, *Klebsiella* spp. can be readily transmitted from one species to another.[8]

Interference with research

Oncology

- *K. oxytoca* has been identified as one of the major *Enterobacteriaceae* species that are increased in cancer cachexia. This bacterium acts as an intestinal pathobiont by altering the gut barrier function in cachectic mice.[32]
- *Klebsiella* 03 lipopolysaccharide was shown to have antitumor activity on allogeneic and syngeneic tumors. A life-prolonging effect on ascites type tumor-bearing mice was reported.[33]

Teratology

- No data

Infectiology / Interactions with other infectious agents

- No data

Immunology

- Colonization of oral *Klebsiella* strains in the intestine drives TH1 cell induction and inflammation.[34]
- Hypervirulent *K. pneumoniae* undermine innate immune processes in the liver and are resistant to Kupffer cell-mediated clearance, which may explain the propensity to cause liver abscesses.[35]
- Bacterial surface structures play an important role in the interaction of *K. pneumoniae* with phagocytes.[36]

Toxicology

- No data

Physiology

- A hypothyroid state increases host resistance, whereas any increase in L-thyroxine decreases resistance in mice infected with *K. pneumoniae*.[37]

Cell biology

- The *K. oxytoca* enterotoxin tilimycin acts as a genotoxin that activates damage repair mechanisms in cultured cells, causes DNA strand breakage and leads to an increased lesion burden in cecal enterocytes of colonized mice.[10]

Assisted reproductive technology

- No data

Special considerations

- Mice and rats experimentally infected with *K. pneumoniae* serve as models for human diseases including pneumonia, endotoxaemia, sepsis, cystitis, pyelonephritis and gastrointestinal colonization.[20,38-40]
- Antibiotic treatment of nude rats resulted in infections with multi-resistant *K. pneumonia*.[41]

Manuel Miller, Munich, March 2022

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