In-vitro virulence and genetic diversity of hypermucoviscous K2 serotype Klebsiella pneumoniae isolates from California sea lions (Zalophus californianus)

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INTRODUCTION

Klebsiella pneumoniae

• Gram-negative, aerobic, non-motile, encapsulated bacilli within the Enterobacteriaceae family.

• Opportunistic pathogen with a broad host range

• In humans a hypervirulent strain has been associated with a hypermucoviscous (HMV) phenotype, but this has not yet been established in animal hosts.

• Multiple capsular serotypes exist within the HMV phenotype, including K1, K2, and K5 serotypes.

• Sudden unexplained increase in the prevalence of HMV K2. K. pneumoniae in marine mammals, especially in California sea lions (Zalophus californianus) (CSLs) – from 0.92% in 2017 to 72% in 2022 (Fig. 1).¹

• Causes klebsielliosis, contributing to disease and standings in marine mammals (Fig. 2. and 3.)

AIMS

1. Investigate the genetic diversity and virulence gene profiles of HMV K2. K. pneumoniae isolates recovered from diseased CSLs in California between 2020 to 2023.

2. Investigate the virulence of representative HMV K2. K. pneumoniae genotypes through in-vitro challenge models of kidney epithelial cells extracted from an African green monkey (Chlorocebus sabaeus) (Vero cell line).

Hypothesis: HMV K2 strains isolated from CSLs are genetically diverse following housekeeping genes and virulence factor typing schemes.

Hypothesis: HMV K2 strains isolated from CSLs are significantly more cytotoxic to mammalian cells (Vero cells) than non-HMV strains recovered from marine mammals.

METHODS

ISOULATE CHARACTERIZATION RESULTS

Fig. 1. Suspected HMV Klebsielliosis admits to The Marine Mammal Center (MMCC).

Evidence of suspected HMV K. pneumoniae admits to The Marine Mammal Center, a marine mammal hospital and rehabilitation facility in Sausalito, CA, from 2017 to 2022. There has been an increase in the number of HMV cases, as well as an substantion of cases earlier in the winter months compared to 2017. Courtesy of Dr. Padraig Duignan and Carlos Rios.

Fig. 2. Lymph node histopathology.

Disseminated bacterial infection with colonization of the lymph node as well as affected southern sea otter (Enhydra lutris nereis). Photo courtesy of Dr. Melissa Miller.

Fig. 3. Polypseudal disease.

K. pneumoniae tissue widespread infection in marine mammals, resulting in conditions such as pleuritis, suppurative peritonitis, and abscesses.

Photo courtesy of Dr. Padraig Duignan

Fig. 4. Isolate Characterization.

K2 serotype clones are distinguished by the presence of the following virulence related genes: luxA (polarisumbilicin), fimH (fimbrial adhesins), esp (type 3 fimbriae promoting adhesion to host epithelial cells), iroD (killing factor), magA (membrane associated type 4 fimbriae). All sequenced isolates had identical Aminoglycoside resistance genes.

Fig. 5. Multiple-PCR.

Multiple-PCR showing all isolates positive for the luxA gene, confirming all isolates were K. pneumoniae. 3/37 isolates were positive for the luxA gene, a regulatory gene for the mucA operon. 2/37 isolates were positive for the K2 capsule serotype. Within the K2 serotype, four different virulence profiles were observed and characterized based on the presence of virulence genes: luxA (polarisumbilicin), fimH (fimbrial adhesins), esp (type 3 fimbriae promoting adhesion for bacterial infection), and espC (type 1 fimbriae promoting adhesion to host epithelial cells). 28 K2 isolates belong to Clade I (luxA+/fimH+/espC+/fimF+). 3 K2 isolates (K2:1, K2:2, K2:3) belong to Clade II (luxA+/fimH+/espC+/fimF+), and 2 K2 isolates (K2:4, K2:5) belong to Clade IV (luxA+/fimH+/espC+/fimF+). The remaining isolates (K1, K3, K4, K5) were not HMV and did not have any K2 serotype isolates (Table 1).

Fig. 6. Isolate Characterization.

K2 serotype clones are distinguished by the presence of the following virulence related genes: luxA (polarisumbilicin), fimH (fimbrial adhesins), esp (type 3 fimbriae), and espC (type 1 fimbriae). All sequenced isolates had identical Aminoglycoside resistance genes following the antomicrobial classes: glycopeptides, rRNA inhibitors, aminoglycosides, quinolones (e. coli EF-Tu), and cephalosporin (P. influenzae PCR).

Fig. 7. Phylogenetic analysis of K. pneumoniae following whole genome sequences and its association with mucoviscosity.

Hypothesis: K. pneumoniae isolates were estimated following low-cost centrifugation of standard bacterial grown in BM broth. Error bars represent standard errors for triplicate samples with triplicate readings for each. Isolates with different levels of virulence indicated significant difference, p values < 0.05, using an ordinary one-way ANOVA analysis.

CYTOTOXICITY RESULTS

Fig. 8. In-vitro Cytotoxicity in Vero cells.

Cytotoxicity of K. pneumoniae to Vero cells was measured quantifying the release of lactate dehydrogenase (LDH) by challenged Vero cells at a multiplicity of infection of 1:100 (Vero cells:bacteria).

• Non-HMV isolates generated significantly greater cytotoxicity when compared to HMV isolates.

• Error bars represent standard errors for triplicate samples with duplicate readings for each. Isolates with different levels of lactate dehydrogenase indicates significant difference, p values < 0.05, using an ordinary one-way ANOVA analysis.

CONCLUSION

Multiple clades exist within the HMV K2 serotype of K. pneumoniae, with genetic diversity in virulence factors, antimicrobial resistance genes, and mucoviscosity.

Investigation of in-vitro virulence of HMV versus non-HMV isolates was hindered by the HMV phenotype’s decreased adhesion capacity to mammalian epithelial cells and faster growth of non-HMV strains.

Further studies exploring virulence should employ invasion assays or in-vivo methods.

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