**Klebsiella pneumoniae** Isolates from Stranded and Wild Caught Marine Mammals: Determination of Prevalence, Phenotype and Genotype

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**Abstract**

*Klebsiella pneumoniae*, a Gram negative, facultative anaerobe belonging to the family *Enterobacteriaceae* is considered an emergent pathogen in both human and veterinary medicine1-3. A novel, invasive strain associated with hypermucoviscosity (HMV) has been recognized in the past 2 decades4, and has been reported in multiple marine mammal species5,6. However, little is known about its epidemiology and pathogenesis in marine mammals7,5. In this study, we swabbed mucosal sites and affected tissues and attempted isolation of HMV *K. pneumoniae* from 270 wild-caught California sea lions (CSL, *Zalophus californianus*) from 3 sampling sites from coastal North America, as well as 336 stranded marine mammals including CSL, pacific harbor seals (PHS, *Phoca vitulina*), northern fur seals (NFS, *Callorhinus ursinus*), and northern elephant seals (ES, *Mirounga angustirostris*). The only isolates recovered were from CSL and we detected a prevalence of 1.5% in stranded CSL and 1.1% in wild caught CSL. To establish baseline data and develop adequate diagnostic methods, we assessed the phenotype and genotype of all HMV *K. pneumoniae* isolates recovered from our sampled animals (n=11), as well as archival isolates from stranded marine mammals (n=19) including HMV and non-HMV isolates. All but 2 of the HMV isolates were capsular type K2 serotype and were positive for the *wyz* gene, while all non-HMV isolates were negative for this serotype and gene. Of the analyzed HMV isolates 24/25 were positive for HMV associated gene *p-rmpA* and 23/25 were positive for *p-rmpA2*. One non-HMV isolate tested positive for these genes. All *Klebsiella* spp. isolates recovered from aquatic animals were identified by *rpoB* and *gyrB* sequence as *K. pneumoniae*, and genetic fingerprinting by repetitive extragenic palindromic PCR showed four discrete clusters demonstrating genotypic variability that loosely correlated with phenotype. Antimicrobial susceptibility testing revealed all our stranded CSL isolates were susceptible to ceftiofur, indicating this antimicrobial is an adequate first choice for treatment of suspected HMV *K. pneumoniae* infections in stranded CSL. To assess the limit of detection of our culture assay, we used swab inoculums from 10 mg/ml suspended fecal solutions and could reliably detect HMV *K. pneumoniae* from concentrations as low as 10^2 CFU/mg of feces. This study shows HMV *K. pneumoniae* infections occur in wild-caught and stranded CSL. Further studies
are needed to assess the epidemiologic dynamics of this pathogen in marine mammals and to understand its potential impact on marine mammal populations.

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