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ORIGINAL ARTICLE

Clinical and bacteriological characteristics of *Klebsiella pneumoniae* causing liver abscess with less frequently observed multi-locus sequences type, ST163, from Singapore and Missouri, US

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Background: *Klebsiella pneumoniae* is the major cause of liver abscesses in several Asian countries. Differences in the type of circulating *Klebsiella* strains and/or the genetic make up of the host seem to be plausible explanations for this.
Methods: Two recent *K. pneumoniae* strains isolated from patients with liver abscess, one from Missouri in the US, and a second one from Singapore, were fully characterized by molecular typing, association of virulent genes, neutrophil phagocytosis, susceptibility to serum killing, and lethality in mice.
Results: Both strains had mucoid colony morphology and were similar in multilocus sequence type (ST-163), drug-susceptibility profile, resistance to phagocytosis and susceptibility to serum killing. Although ST-163 is a single nucleotide variant (SNV) to the major ST-23, which is specific to serotype K1 *K. pneumoniae* that causes liver abscess in Taiwan, these two isolates differ in capsular serotype. One was serotype K1 and the other K29. Since a serotype K35 with ST163 was reported previously to cause peritonitis, serotype K29 with SNV to ST-23 was not impossible. Pulsed field gel electrophoresis by XbaI digestion showed different restriction patterns. The virulence-associated genes *rmpA* and *aerobactin* were only present in the serotype K1 isolate from Singapore and not in the serotype K29 isolate from Missouri. The serotype K1 isolate was also more virulent to mice.

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Conclusion: The reasons underlying the high prevalence of ST-23 or its SNV in *K. pueumonaie* liver abscesses is worth further investigation.

Introduction

Klebsiella pneumoniae is one of the major causes of pyogenic liver abscess. In Taiwan, over 80% of bacterial liver abscesses are caused by *K. pneumonia*.[1] In Singapore and South Korea, similar observations have been made.[2,3] Most patients with a *K. pneumoniae* liver abscess display bacteremia and septic metastatic complications, including endophthalmitis and meningitis. Comorbid conditions such as diabetes mellitus and alcoholism are associated with increased risk of *K. pneumoniae* liver abscess.[1,2,4]

Among reports of *K. pneumoniae* liver abscess, the vast majority of patients are Asian. Case reports from other countries including the US have indicated that most patients with *K. pneumoniae* liver abscess were of Asian descent.[5] However, it is not known whether these *Klebsiella* isolates from Asians living abroad are similar to isolates predominantly circulating in Asia. In Asia, serotype K1 is the majority capsular serotype in patients with liver abscess, followed by serotype K2.[1–3] Other virulence-associated, genes such as *rmpA* and *aerobactin*, re common in isolates.[6] Turton et al[7] found that sequence type ST23 was the major sequence type (ST) in *K. pneumoniae* liver abscess isolates and these are specifically associated with serotype K1. To our knowledge, there are no similar studies on the detailed characterization of *K. pneumoniae* isolates from liver abscess patients from countries other than those in Asia. This could be partly because of the very limited number of cases in other countries. This study was conducted to fully characterize two recent isolates from the US and Singapore based on clinical as well as bacteriological features including serotyping, antimicrobial susceptibility, genotyping, virulence evaluation and 50% lethal dose (LD_{50}) in mice.

Methods and materials

Selected *K. pneumoniae* liver abscess isolates

The isolates for this study were selected by an international collaboration group for *K. pneumonaie* liver abscess study. Liver abscess was diagnosed by ultrasonography or computed tomography (CT) and confirmed by culture. Identification of the isolates was according to standard clinical microbiologic methods. The two isolates in this study were obtained from Singapore and the US. One hypervirulence *K. pneumoniae* liver abscess isolate with serotype K1 from Taiwan[8] was included as a control for virulence assessment.

Serotyping

Isolates were serotyped using capsule swelling reaction with antisera obtained from the Health Protection Agency in the UK. In addition, one tube multiplex polymerase chain reaction (PCR) for K1, K2 and K5 was performed according to a previously published method.[9]

Antimicrobial susceptibility

Antimicrobial susceptibility was determined by microbroth dilution and disk diffusion according to the Clinical and Laboratory Standards Institute (CLSI) method. The following antimicrobial agents were used: ampicillin, cefazolin, cephalothin, amoxicillin/clavulanic acid, cefoxitin, cefotaxime, ceftazidime, aztreonam, imipenem, amikacin, gentamicin, ciprofloxacin and trimethoprim/sulfamethoxazole.[10] All drugs were incorporated into the Mueller-Hinton broth (TREK Diagnostic System Ltd, West Sussex, UK) in serial twofold concentrations from 0.025 to 64 mg/L. Two control strains, *Escherichia coli* ATCC 35218 and ATCC 25922, were included in each test run. Inoculated plates were incubated at 35°C for 16 to 18 h. The minimal inhibitory concentration (MIC) of each antimicrobial agent was defined as the lowest concentration that inhibited visible growth of the organism.

PCR for rmpA and aerobactin genes

PCR was used to determine the presence of the *rmpA* and *aerobactin* genes.[6] An overnight-cultured bacterial colony was added to 300 μl of water and boiled for 15 min to release the DNA template. Previously published primers used for PCR are listed in Table 1. The reaction mixture was kept at 95 °C for 5 min, followed by 40 temperature cycles of 95 °C for 1 min, 50 °C for 1 min, 72 °C for 2 min, and 72 °C for 7 min. The expected PCR product of *rmpA* was 535 bp in length.

Multilocus sequence typing (MLST)

Multilocus sequence typing was performed according to Turton et al.[7] Sequences of seven housekeeping genes were obtained for isolates from liver abscess patients and carriers. Sequence information was compared with that on the MLST web site (www.pasteur.fr/mlst/) developed by Keith Jolley. Alleles and STs were assigned accordingly. Sequences of any alleles that were not on the database were submitted to the curator and new allele numbers were obtained. Strains with a difference in two or more alleles were considered to be unrelated.

Pulsed field gel electrophoresis (PFGE)

Total DNA was prepared and PFGE was performed as described previously.[3] The restriction enzyme *Xba*I (New England Biolabs, Beverly, MA, USA) was used at the temperature suggested by the manufacturer. Restriction fragments were separated by PFGE in 1% agarose gel