Multilocus sequence typing analysis of *Klebsiella pneumoniae* isolated from swine

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ABSTRACT

A total of 86 strains *Klebsiella pneumoniae* isolated from swine were collected to analysis the molecular genetic characteristic by multilocus sequence typing (MLST). The eURST software was used to evaluate the results of MLST. The results indicated that all isolates clustered into 14 ST types(STs). ST106 (including 3 strains) and ST23 (including 2 strains) were single branch. Other 12 ST types belong to the CC258 clonal complex. This study showed that *klebsiella pneumoniae* isolated from swine in this region has some genetic diversity and the main types were ST258 clone complex, it may be transfered between swine and human.

Key words: *Klebsiella pneumoniae*, Multilocus sequence typing, Swine.

INTRODUCTION

*Klebsiella pneumoniae* (*K pneumoniae*) belongs to the family *Enterobacteriaceae* and widely exist in skin, respiratory, alimentary canal of animal and human as well as all environments (Seidler et al.,1975; Ullmann et al.,1998; Gregg et al.,2016). *K pneumoniae* is an important opportunistic pathogen that can cause different infections, including pneumonia, meningitis, bloodstream infections, urinary tract infections, surgical or wound site infections, and septicemia (Keynan et al., 2007; Kim et al., 2009; Ben et al., 2012; Munoz et al., 2013; De et al.,2015; ). So *K pneumoniae* is one of the main pathogenic bacteria causing nosocomial infection. With the widely use of antibiotics in recent years, *K pneumoniae* has produced resistance to many antimicrobial agents. Especially the strains of multidrug-resistant (MDR), hyper-virulent strains and pandrug-resistant (PDR) bacteria have been isolated from patients (Magiorakos et al.,2012; Shon et al.,2013; Giamarelou et al.,2013), which brought the new challenge for the treatment of infectious diseases caused by *K pneumoniae*.

There are many studies on drug-resistant, virulent gene and molecular epidemiology of *K pneumoniae* isolated from clinic in human (Cuzon et al., 2010; Samuelsen et al.,2011; Holt et al., 2015). But in the veterinary field *K pneumoniae* is considered as an opportunistic pathogen and doesn’t usually cause disease among animals. Although some research has reported *K pneumoniae* can cause many infectious diseases in animals (Brisse et al.2005; Locatelli et al.2010). To our knowledge, there is little research on molecular characteristic of *K pneumoniae* isolated from animals.

The aim of the present study was to investigate molecular genetic characteristics of *K pneumoniae* isolated from swine. A further aim was to assess the epidemiological relatedness of *K pneumoniae* between swine and human. We analysis the molecular epidemiology of *K pneumoniae* isolated from swine by multilocus sequence typing (MLST).

MATERIALS AND METHODS

Isolation and identification of bacterial strains: From November 2012 to December 2014, a total of 86 non-duplicate *K pneumoniae* strains isolated from veterinary hospital and large-scale pig farms were collected in HeNan province, China. All the isolates were preliminary identified by morphology, Gram stain and biochemical test, then *K pneumoniae* isolates were final identified by a Vitek-AMS60 microbiology analyzer (bioMérieux, Marcy Etoile, France) in accordance with the manufacturer’s instructions. *Escherichia coli* ATCC 25922 and *K pneumoniae* ATCC700603 were used as the control strains.

DNA extraction and PCR amplification housekeeping gene: Bacterial DNA was extracted from all isolates of *K pneumoniae* using a DNA extraction kit (DP302, Tian Gen Corporation, China) or genomic DNA purification kit (Tian Gen Corporation, China). The final DNA concentration was determined by recording its optical density at 260 and 280 nm, respectively, using a NanoDrop ND-1000 spectrophotometer. MLST of *K pneumoniae* was performed based on seven housekeeping genes: *rpoB*, *bgap*, *omdh*, *pgi*, *pgI*, *phoE* and *tonB*, as described previously (Diancourt et al., 2005). The
### RESULTS AND DISCUSSION

A total of 14 unique STs were identified from the 86 isolates, the ST258 was high prevalence contained 23 strains (26.74% of the total 86 isolates), followed by ST1461 contained 12 strains (13.95%), ST855 contained 9 strains (10.47%) and ST11 contained 7 strains (8.14%). The ST23 and ST1680 were least and only contained 2 strains (Table 1).

Assignment of the STs to lineages established that 2 STs (ST106 and ST23) were both unique and unrelated to any others. The remaining isolates were assigned into 1 clonal complex (CC258), this CCs contained 12 members, and ST258 was predicted founder in this CCs (fig 1). ST1461, ST554, ST512 were double-locus variant (DLV), allelic gene gapA and tonB were different (Table 1).

The allelic profile (STs) in this study was compared with pubMLST database. The results showed that CC258 was in the central region of the graph, clustered one group independently. But ST23 and ST106 was single branch respectively (fig 2).

#### Table 1: The MLST analysis of K pneumoniae isolated from swine

<table>
<thead>
<tr>
<th>ST type</th>
<th>number of strains</th>
<th>Allele gene</th>
<th>SLV</th>
<th>DLV</th>
<th>TLV</th>
<th>Average distance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>gapA infB mdh pgi phoE rpoB tonB</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>7</td>
<td>3 3 1 1 1 1 4</td>
<td>9</td>
<td>2</td>
<td>0</td>
<td>1.18</td>
</tr>
<tr>
<td>258</td>
<td>23</td>
<td>3 3 1 1 1 1 79</td>
<td>7</td>
<td>4</td>
<td>0</td>
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</tr>
<tr>
<td>270</td>
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<td>3 3 1 1 1 1 23</td>
<td>5</td>
<td>6</td>
<td>0</td>
<td>1.54</td>
</tr>
<tr>
<td>855</td>
<td>9</td>
<td>3 3 1 1 1 1 26</td>
<td>5</td>
<td>6</td>
<td>0</td>
<td>1.54</td>
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<tr>
<td>572</td>
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<td>0</td>
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<tr>
<td>1680</td>
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<td>5</td>
<td>6</td>
<td>0</td>
<td>1.54</td>
</tr>
<tr>
<td>1461</td>
<td>12</td>
<td>18 3 1 1 1 1 79</td>
<td>3</td>
<td>6</td>
<td>2</td>
<td>1.90</td>
</tr>
<tr>
<td>554</td>
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<td>3 3 1 1 83 1 1 4 1 6 4 2.27</td>
<td></td>
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<tr>
<td>106</td>
<td>3</td>
<td>4 1 1 1 1 1 9   -   -   -   -</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>2</td>
<td>2 1 1 1 1 9   4 12 -   -   -   -</td>
<td></td>
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</tr>
</tbody>
</table>

Note: SLV single-locus variants, DLV double-locus variants, TLV triple-locus variants

### MLST analysis: Sequence types were determined at the K pneumoniae MLST websites, http://bigdb.web.pasteur.fr/klebsiella/klebsiella.html. The seven sequences were aligned with the order of gapA-infB-mdh-pgi-phoE-rpoB-tonB. Each isolate was therefore designated by seven numbers, constituting a distinct allelic profile or STs. The STs were grouped into lineages or clonal complexes using the program eBURST V3. The members of a lineage were defined as groups of two or more independent isolates with an ST that shared identical alleles at four or more loci. Each lineage was named after the ST identified as the putative founder of the group by BURST, followed by the word “complex”. Two different STs sharing six of the seven loci constituted a single-locus variant (SLV). A double locus variant (DLV) contained two STs differing in two loci and other loci should be identical. A triple-locus variant (TLV) included two STs differing in three loci. A clonal complex was composed of at least three STs with only SLVs. Only two STs belong to the same group with SLV was called doublet. The remaining STs, which had no SLV with other STs, were termed singletons. The founders (ancestry types) of CCs were predicted with 1,000 re-samplings for bootstrap.
Figure 1: Distribution of ST type of *K pneumoniae* isolated from swine

Note: Each dot represents one ST type, the size of dot represents the number contained, the length represents the possible evolutionary. All ST types in box belongs one clone complex is the most popular one. MLST is a nucleotide sequence-based method that is adequate for characterizing the genetic relationships among bacterial isolates (Laure *et al*., 2005). It provides unambiguous and portable data that allow the implementation of multiuser international databases. In present study, MLST was applied to characterize diversity and epidemiology of *K pneumoniae* isolates from swine.

In this study, a total of 14 STs were collected from 86 isolated stains. Except for 2 single types, all STs clustered into one group (CC258). The molecule genetic types of *K pneumoniae* prevalence in local swine farms were simplex. ST258 is widely popular in the world, some research reports of ST258 epidemic in USA, Hungary, Korea, Malaysia and Singapore (Roh *et al*., 2011; Jain *et al*., 2013; Brinkworth *et al*., 2015). There was seldom document about ST258 epidemic in China. Our research showed that main prevalence strains is ST258 in different swine farms. So we speculate that *K pneumoniae* spread without strict host specificity and can transmit between human and swine. Some research report ST11 was main prevalence type of *K pneumoniae* in Chinese hospital (Qi *et al*., 2011; Yang *et al*., 2013). In this study we found 7 strains isolated from swine belongs ST11, which proved that ST11 may infection both swine and human.

Have ST258 and ST11 widely been popular among swine in China? We will investigate this by increasing the sample numbers in future. Meanwhile, the resistance to antibiotics of different STs of *K pneumoniae* should been detected to find the relations between genetic type and drug-resistant phenotype, which will offer a possible strategy for control *K pneumoniae* spread among animals and humans.

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REFERENCES


