Comparative Genomic Analysis and Phenotypic Characterization of Bronchoscope-Associated Klebsiella Aerogenes

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Research

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Abstract

**Background:** Bronchoscopes has been linked to the outbreaks of nosocomial infections. We aim to investigate the phenotypic and genomic profiles of bronchoscope-associated *Klebsiella aerogenes* isolates, and their association with genome public available isolates from human and environment.

**Methods:** We performed a prospective single-center study sampling echoendoscopes after clinical use and after normal decontamination procedures. Bacterial screening was conducted by culturing the sample on Mueller-Hinton agar plates. Antimicrobial susceptibility testing was performed using the broth microdilution method. Whole-genome sequencing of *K. aerogenes* isolates was performed using an Illumina HiSeq system and comparative genomics analysis were conducted.

**Results:** Over the 5-month period, a total of 358 isolates and 13 isolates were recovered from samples after clinical procedures and samples after decontamination procedures, respectively. Antimicrobial susceptibility testing found 7 *K. aerogenes* isolates to exhibit low-level resistance to antimicrobial agents. Among 7 *K. aerogenes* isolates, we found 5 sequence types (STs). Whole genome sequencing and comparison analysis observed the genetic diversity in our bacterial collection, which clustered into three main clades. Furthermore, we identified a total of 43 antimicrobial resistance genes in the *K. aerogenes* core genomes. As expected, human isolates encoded more antimicrobial resistance genes than that environmental isolates.

**Conclusions:** This study first described the phenotypic and genomics characteristics of bronchoscope-associated *K. aerogenes*. The present observations demonstrated that broadly investigation of specific pathogens using publicly available global genomes offered the opportunity to identify prevalent clones associated with various hosts, sources, and geographical locations.

**Background**

Flexible bronchoscopes are the medical devices widely used for diagnostic and therapeutic procedures. Usually they are heat labile and complex, which lead to difficult to clean [1]. Patient-ready reusable, flexible bronchoscopes were contaminated and damaged, and pose a serious threat to patient safety [2]. It is well documented that some nosocomial outbreaks have occurred linked to contaminated or inadequately disinfected bronchoscopes in literature [3, 4]. Moreover, recent studies determined that microbial transmission occurred even when proper cleaning and disinfection protocols are followed with standard guidelines [5, 6]. Recently, endoscopes have been suggested as point sources of nosocomial Enterobacteriaceae infections [7, 8]. Previous investigations have shown significant reduction of microbial contaminants with cleaning alone and recommendations require cleaning to be performed promptly following use [5, 9, 10]. In China, the microbiology data onto cleaning and disinfection of endoscopes is unclear. Moreover, bronchoscope-associated microbial profiles and phenotypic characteristics are largely unknown.
Klebsiella is a gram-negative, rod-shaped, anaerobic bacterium, which is a commensal microorganism living in the mouth and gut. However, Klebsiella is now resistant to many antimicrobial agents including one of the latest antibiotics-carbapenem, which represents a serious challenge to the public health [11, 12]. Klebsiella aerogenes belongs to the genus Klebsiella. It has been documented as important opportunistic and multi-resistant bacterial pathogens for patients during the past decades in clinical settings [13–15]. It is widely associated with bloodstream, skin and soft tissue, respiratory, and urinary tract infections [16]. Recently, the emergence of carbapenem-resistant K. aerogenes and colistin-resistant K. aerogenes isolates in China is of concern [17–20]. However, the genetic background of K. aerogenes isolates recovered from clinical settings in China remains largely unknown.

We hypothesized that bacterial colonization of medical instruments may associate with the clinical transmission of multidrug-resistant (MDR) K. aerogenes isolates in clinical settings. The aims of the current study were to evaluate the bronchial bacterial colonization in the disinfection and sterilization center, and to investigate the phenotypic characteristics and genomic complexity of of K. aerogenes strains isolated from bronchoscope samples.

Material And Methods

Setting

From January 2019 to May 2019, the study was conducted in the Disinfection & Sterilization Center of the First Affiliated Hospital, School of Medicine, Zhejiang University, where both gastrointestinal and respiratory endoscopes are reprocessed. During the study period, procedures in our institution were performed using bronchoscopes (model BF260) (Olympus, Japan). The cleaning of bronchoscopes was carried out with an enzymatic detergent solution, endozyme. Manual disinfection was performed by soaking the device into 2% glutaraldehyde.

Sampling

Samples were collected under aseptic conditions from bronchoscopes following clinical procedures and after usual decontamination procedures by flushing thoroughly with 10 mL of sterilized phosphate buffered saline (PBS) as described previously [21]. Collected samples were put in cool-boxes with ice-packs (4-8°C) upon collection and transported in 4 hours to laboratory.

Bacterial isolation and identification

All samples were plated on Mueller-Hinton agar plates (Oxoid, UK) using the sterile swab. The agar plates were incubated for 18–24 hours at 37°C. Single colony was selected from each species per sample. All of the positive cultures were selected for identification. Bacterial identification was conducted by matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS) (Bruker, Leipzig, Germany), and further checked by PCR and sequencing.

Antimicrobial susceptibility testing
The minimum inhibitory concentrations (MICs) of 9 *K. aerogenes* isolates was determined using the agar dilution method according to the Clinical and Laboratory Standards Institute (CLSI) standards [22]. 19 antimicrobials were tested as described previously [23]. Antimicrobial susceptibility testing for colistin and tigecycline were performed by the microbroth dilution method as described by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (http://www.eucast.org/). The MIC results were interpreted using the CLSI standards (Third Edition: M45).

**Whole genome sequencing (WGS) and *in silico* analysis**

WGS was performed on all *K. aerogenes* strains identified in this work.

The extracted genomic DNA was evaluated by agarose gel electrophoresis. The concentration and purity of genomic DNA were determined using NanoDrop 2000 (Thermo Scientific, Waltham, USA) and Qubit® version 2.0 fluorometer (Thermo Scientific), respectively. The sequencing library was prepared by using Illumina Nextera XT kit (Illumina, San Diego, USA). A-tailed fragments were ligated with paired-end adaptors and PCR-amplified with a 500-bp insert. WGS was performed with an Illumina NovaSeq 6000 platform (Novogene Co., China). PCR adapter reads and low-quality reads from the paired-end and mate-pair library were filtered using in-house pipeline. Paired reads were then assembled into a number of scaffolds using Velvet version 1.2.10 [24]. Multilocus sequence typing (MLST) analysis was performed as described previously [25]. ARGs were identified using the ResFinder 2.1 database [26].

**Phylogenetic analysis of *K. aerogenes* isolates**

To further characterized the evolutionary relationship among *K.aerogenes* isolates, we created a core genome-based phylogenetic tree using 7 *K. aerogenes* genomes sequenced in this study and 51 randomly selected publicly available *K. aerogenes* genomes (Table S1). The isolate collection includes strains from humans (n = 44), the environment (n = 9) and other sources (n = 5) that were widely distributed over time and geographical locations. All collection genomes were annotated using Prokka [27] and RAST tool [28]. The core genes in the genomes of *K. aerogenes* genomes were identified using Prokka [27] and Roary [29]. Maximum likelihood-based phylogenetic reconstruction was performed with RAxML version 8.2.10 using generalized time reversible (GTR) + Γ nucleotide substitution model [30]. One hundred bootstrap replicates were evaluated to determine branch support. A maximum-likelihood phylogenetic tree based on the core single nucleotide polymorphism alignments was generated using FastTree [31]. Phylogenetic tree visualizations were produced using the Interactive Tree of Life (https://itol.embl.de/).

**Results And Discussion**

**Bacterial isolation and identification**

Over the 5-month period, 250 bronchoscopes were sampled and 500 samples were collected, including 250 samples after clinical procedures and 250 samples after usual decontamination procedures. All bronchoscope samples were tested for bacterial screens. A total of 358 isolates and 13 isolates were
recovered from samples after clinical procedures and samples after decontamination procedures, respectively (Table S2 and Table S3). This might explain by the low-level contamination with environmental and skin bacteria, since bronchoscope samples were collected after the clinical procedures without any disinfection or cleaning processes. Of note, most of the detected microorganisms were Gram-positive bacteria, such as Staphylococcus epidermidis (n = 69), Streptococcus salivarius (n = 42), and Streptococcus oralis (n = 23). Among the Gram-negative bacilli isolates, most of them were that belong to the Enterobacteriaceae group (Table S2 and Table S3). Outbreaks and pseudo-outbreaks associated with bronchoscopes have been well documented in the literature [32]. These nosocomial infections commonly associated with Mycobacterium spp. and Enterobacteriaceae isolates [33-37]. Moreover, K. aerogenes was found in both groups (before and after the cleaning procedures). All this prompted us to further investigated the phenotypic and genomic characteristics of 7 K. aerogenes identified in this work.

**AST and MLST of K. aerogenes isolates**

AST results of 7 K. aerogenes isolates were detailed in Table 1. The full resistance rate was observed for amoxicillin-clavulanic acid and cefoxitin (100%). All the isolates were susceptible to piperacillin-tazobactam, cefuroxime, ceftazidime, ceftriaxone, cefoperazone-sulbactam, cefepime, ertapenem, amikacin, levofloxacin, tigecycline, trimethoprim-sulfamethoxazole. Interestingly, 5 isolates were intermediate to imipenem. Among 7 K. aerogenes isolates, we found 5 sequence types (STs), which were ST135 (n = 2) and ST1358 (n = 2), followed by ST1357 (n = 1), ST1359 (n = 1), and ST1363 (n = 1). K. aerogenes is reported to associated with nosocomial infections and displaying multidrug resistance [16], and the most prevalent STs were ST93 and ST4 [38]. However, we didn't detect any multidrug resistant K. aerogenes in this work. Furthermore, STs of K. aerogenes found in this work have not been described in the literature. These data suggested that these colonized isolates may have an environmental origin.

**Whole genome sequencing and comparison analysis**

Roary matrix-based gene sequence analysis generated a large pan-genome of 18,105 gene clusters of 58 full genomes. The whole-genome phylogeny (Figure 1) revealed a population structure that was generally concordant with MLST (data not shown). Genetic diversity was observed in our bacterial collection, which clustered into three main clades. The 2 ST1358 strains identified in this study were aggregated in 1 clade with 1 ST1364 human isolate from Spain, which suggested that ST1358 and ST1364 might originate from the same ancestor. The results also indicate that 11 ST93 isolates and 9 ST56 were clustered into one separate sub-cluster, respectively, which exhibited a slight difference in the core genome sequence. Recent studies found that ST93 was the most prevalent clone in the global K. aerogenes genome database, which indicated that ST93 might be dominant sequence type global clones in clinical settings [13, 39]. Furthermore, the emergence of fecal carriage and human infection MDR K. aerogenes isolates resistant to multiple antibiotics, especially resistant to carbapenems is considered a substantial threat to public health [17, 40]. Although phenotypic and genomic evidence from the current study revealed that isolates recovered in this work are not multi-resistant, active surveillance of bronchoscope-associated K. aerogenes isolates would improve our understanding on the population structure of this species. Of note,
all isolates recovered from this study have a close relation to environmental or human isolates. A recent study investigated the population structure, virulence, and antimicrobial resistance in K. aerogenes [38]. Their findings showed that K. aerogenes has an open pangenome and a large effective population size, which is in line with our results.

**Resistant determinants of K. aerogenes isolates**

We identified a total of 43 antimicrobial resistance genes in the K. aerogenes core genomes (Figure 2). The resistome of K. aerogenes comprise a high number of antibiotic efflux pumps as well as narrow and extended spectrum β-lactamases. As expected, human isolates encoded more antimicrobial resistance genes than those environmental isolates. Of note, 3 isolate from this work possessed only one resistance gene, fosA, which is consistent with phenotypic characteristics.

**Conclusion**

In this study, we described the phenotypic and genomics characteristics of bronchoscope-associated K. aerogenes, although it is limited by the relatively small number of strains identified and which are not multi-resistant. The expanding of K. aerogenes in the surveyed disinfection and sterilization center may associate with the environmental transmission. These results may lead to a better understanding of genetic background and population structure of K. aerogenes.

**Declarations**

**Ethics approval and consent to participate**

Not applicable

**Consent for publication**

Not applicable

**Accession numbers**

All genome assemblies of K. aerogenes isolates were deposited in GenBank and are registered under BioProject accession no. PRJNA633774.

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Competing interests

All the authors reports no conflicts of interest in this work.

Authors’ contributions

Each author is expected to have made substantial contributions to the conception.

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**Figures**
Figure 1

Maximum-likelihood phylogeny of 58 representative global K. aerogenes isolates. The trees were constructed using Roary software. The tips of branches are colored according to hosts, countries, and sources. Red words indicate the 8 strains in this study.
Figure 2

Antimicrobial resistance genes identified in the genomes of K. aerogenes isolates by analyzing the WGS data. The antimicrobial resistance genes (ARGs) are shown on the bottom. Yellow indicates the presence of the ARGs, and blue indicates the absence of the ARGs.

Supplementary Files

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