Characterization of Bordetella Bronchiseptica Isolated from Rabbits in Fujian, China

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Research article

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Abstract

Background *Bordetella bronchiseptica* can infect many animal species, and is a potential zoonotic pathogen that can also infect humans. In rabbits, infection of *B. bronchiseptica* is associated with respiratory disease, which causes economic losses to the rabbit farming. Fujian Province is a traditional importance rabbit farming area in China. However, no literature about the epidemiology and characteristics of *B. bronchiseptica* in rabbits in Fujian Province has been reported.

Results A total of 219 *B. bronchiseptica* isolates were recovered from the 833 lung samples of dead rabbits with respiratory disease. The 219 isolates were typed into 11 sequence types (STs) including 5 known STs (ST6, ST10, ST12, ST14 and ST33) and 6 new STs (ST88, ST89, ST90, ST91, ST92 and ST93) by using multi-locus sequence typing (MLST). Surprisingly, all the 219 isolates carried the 5 virulence genes of *fhaB*, *prn*, *cyaA*, *dnt* and *bteA* in the PCR screening. Moreover, the isolates resistance to cefixime, ceftizoxime, cefatriaxone and ampicillin were detected, and the resistance rates to the 4 kinds of drug were 33.33, 31.05, 11.87 and 3.20%, respectively.

Conclusions In the present study, we showed for the first time that *B. bronchiseptica* is widespread in rabbits in Fujian Province, and that *B. bronchiseptica* is an important pathogen associating with respiratory disease in rabbits in Fujian Province. Moreover, it should be alert to the potential occurrence of transmission events between rabbits and humans because the *B. bronchiseptica* strain of ST12 that can infect humans were also isolated from rabbits in Fujian Province.

Background

B. bronchiseptica can infect a number of animal species [1]. The infection of the pathogen is primarily associated with respiratory diseases, such as canine infectious respiratory disease syndrome in dogs, tracheobronchitis in cats, atrophic rhinitis in pigs, pneumonia in horses and snuffles in rabbits [2–6]. Importantly, reports showed that humans could also be infected with *B. bronchiseptica* by exposure to infected rabbits and cats, highlighting the zoonotic potential of the pathogen [7, 8]. Therefore, more efforts should be endeavored to understand the epidemiology and characteristics of *B. bronchiseptica* in animals, which will help in preventing the transmission of *B. bronchiseptica* from animals to humans.

B. bronchiseptica spreads rapidly in rabbits and the infection is often persistent, causing economic losses to the rabbit farming [9]. Fujian Province, in the southeast of China, is one of the important rabbit farming areas in China [10]. To the best of our knowledge, *B. bronchiseptica* is a common pathogen isolated from rabbits with respiratory disease in Fujian Province. However, the knowledge of the epidemiology and characteristics of the *B. bronchiseptica* in rabbits in Fujian province is largely unknown. In this study, *B. bronchiseptica* strains were recovered from the lung samples of dead rabbits with respiratory disease. The isolates were then characterized by defining the STs, screening the virulence genes and testing the antimicrobial susceptibility.

Results

B. bronchiseptica isolation and identification

Two hundred and nineteen *B. bronchiseptica* strains were isolated from the 833 lung samples of dead rabbits with respiratory disease. The *B. bronchiseptica* strains could be recovered from the all 9 rabbit farms and the all 4 rabbit breeds (Table 1). The infection rates of *B. bronchiseptica* in the 9 rabbit farms and the 4 rabbit breeds ranged from 13.79 to 38.42% and 15.66 to 50.24%, respectively.

Table 1
Distribution, origin and STs of the 219 *B. bronchiseptica* isolates

Cities	Rabbit farms	Rabbit breeds	No. of samples	No. of isolates	ST:No. of isolates
Fuzhou	Farm A	Fujian Yellow rabbit	177	68	ST12:19; ST14:34 ST33:12; ST89:***2*** ST93:***1***
	Farm B	Fujian Yellow rabbit	113	37	ST6:12; ST14:8 ST33:15; ST90:***2***
Longyan	Farm C	Fujian White rabbit	91	22	ST6:8; ST10:7 ST33:6; ST88:***1***
	Farm D	Fujian White rabbit	102	21	ST12:14; ST14:6 ST91:***1***
	Farm E	Fujian White rabbit	68	15	ST10:6; ST33:9
	Farm F	Minxinan Black rabbit	75	18	ST14:3; ST33:13 ST90:***2***
	Farm G	Minxinan Black rabbit	79	18	ST6:7; ST33:10 ST92:***1***
Nanping	Farm H	Hyplus rabbit	87	12	ST12:3; ST14:8 ST33:1
	Farm I	Hyplus rabbit	41	8	ST6:6; ST10:2
Total			833	219	

The bold-italic characters indicate the new STs.

MLST analyses

The results of MLST analyses showed that the 219 *B. bronchiseptica* strains were typed into 11 STs, with the ST33 (30.14%, 66/219), ST14 (26.94%, 59/219) and ST12 (16.44%, 36/219) were the 3 most predominant STs. Among the 11 STs, six new STs were detected including ST88, ST89, ST90, ST91, ST92 and ST93 (Table 1), and the MLST profiles of the six new STs had been submitted to the PubMLST database. The 7 house-keeping genes of each of the isolate of the 11 STs were concatenated for phylogenetic analysis. A maximum likelihood tree was constructed (No. of bootstrap replications was 1000) by using the MEGA 5.0 software. The 11 STs were mainly grouped into 2 clusters, cluster I and cluster II. Cluster I contained 9 STs including ST6, ST10, ST12, ST14, ST33, ST88, ST89, ST90 and ST92, whereas cluster II only contained 2 STs including ST92 and ST93 (Fig. 1).

Virulence genes detection

Five virulence genes were screened in the 219 *B. bronchiseptica* isolates. The results showed that all the 5 virulence genes of *fhaB*, *prn*, *cyaA*, *dnt* and *bteA* were positive in the all 219 isolates.

Antimicrobial susceptibility test

The results of antimicrobial susceptibility test showed that most of the *B. bronchiseptica* isolates in this study were susceptible or intermediate susceptible to streptomycin, ciprofloxacin, gentamycin, ofloxacin, kanamycin and norfloxacin, and the sensitive rates to the 6 kinds of drug were 100, 99.09, 98.63, 96.80, 96.34 and 21 %, respectively (Table 2). The isolates resistant to cefixime, ceftizoxime, cefatriaxone and ampicillin were observed, and the resistance rates to these 4 kinds of drug were 33.33, 31.05, 11.87 and 3.20 %, respectively (Table 2). No isolate of multi-drug resistant was detected.

Table 2

Antimicrobial susceptibility test of the 219 *B. bronchiseptica* isolates

Antibiotics	No. of isolates			Percentage of resistance or sensitive	
	R	I	S	Resistance rate (%)	Sensitive rate (%)
Ampicillin	7	210	2	3.20	0.91
Cefixime	73	145	1	33.33	0.46
Cefatriaxone	26	185	8	11.87	3.65
Ceftizoxime	68	151	0	31.05	0
Gentamycin	0	3	216	0	98.63
Kanamycin	0	8	211	0	96.34
Streptomycin	0	0	219	0	100
Ofloxacin	0	7	212	0	96.80
Ciprofloxacin	0	2	217	0	99.09
Norfloxacin	0	173	46	0	21

“R” represents resistance; “I” represents intermediate; “S” represents susceptible

Table 3

Primers used for amplification of the 5 virulence genes in the *B. bronchiseptica* isolates

Genes	Descriptions	Primer sequence (5’-3’)	Product size (bp)	Accession numbers of reference genes
fhaB	Filamentous hemagglutinin	F:gcgcagaacatcaccaatg	475	CP024173 (Rang from 3041205 to 3042932)
		R:tgaaatactccatggcggac		
prn	Pertactin	F:gacctcgctcagtcgatc	555	AJ245927
		R:gaagacattcatgcggaacag		
cyaA	Adenylate cyclase-hemolysin toxin	F:ctacgagcagttcgagtttc	377	Z37112
		R:tattcatgtcgccgtcgta		
dnt	Dermononecrotic toxin	F:tgatcctgcagtggttgatc	491	U59687
		R:atcggcatacgccagatc		
bteA	*Bordetella* type-III secretion system effector A	F:tgttgagcaacaacgtcaatc	474	HE974463
		R:tatgcaggtcttcgaggttc		

Discussion

The present study described the epidemiology and characteristics of *B. bronchiseptica* in rabbits in Fujian Province. The results showed that *B. bronchiseptica* was prevalent in the 9 rabbit farms and the 4 rabbit breeds, and the infection rate of *B. bronchiseptica* in rabbits in Fujian Province was as high as 26.29% (219/833). A previous study showed that *B. bronchiseptica* infection alone was not significantly associated with respiratory disease in rabbits [6]. In the present study, except the 131 (59.82%, 131/219) *B. bronchiseptica* isolates co-infecting with *P. multocida*, *E. coli* or *K. pneumoiae*, the other 88 (40.18%, 88/219) isolates were the only etiologic agent isolated from the lung samples of dead rabbits with respiratory diseases. It is deduced that *B. bronchiseptica* is an important pathogen causing respiratory diseases in rabbits in Fujian Province. The results also showed that the infection rate of *B. bronchiseptica* in the foreign rabbit breed (Hyplus rabbit) was lower than that of the 3 local rabbit breeds (Fujian Yellow rabbit, Fujian White rabbit and Minxinan Black rabbit). It is thought that the all-in all-out system used in the production of the foreign rabbit breed (Hyplus rabbit) might contribute to the lower infection rate.

The 219 *B. bronchiseptica* isolates in this study were typed into 5 known STs (ST6, ST10, ST12, ST14 and ST33) and 6 new STs (ST88, ST89, ST90, ST91, ST92 and ST93). In the PubMLST database (https://pubmlst.org/bordetella/), *B. bronchiseptica* strains of ST6 are isolated from rabbits in Switzerland, strains of ST10 are isolated from rabbits in USA, strains of ST12 are isolated from rabbits in USA and Denmark, and strains of ST14 are isolated from rabbits in USA and UK. However, strains belonging to ST33 that the most prevalent ST (30.14%, 66/219) in rabbits in Fujian Province are isolated from seal, dog, leopard and horse, but not from rabbits. Notably, strains of ST12 could also be isolated from humans, suggesting the potential zoonotic transmission between rabbits and humans [7].

Expression of the virulence factors facilitates the invasion of *B. bronchiseptica* in hosts [11]. Surprisedly, all the 219 *B. bronchiseptica* isolates in this study carried the 5 screened virulence genes of *fhaB*, *prn*, *cyaA*, *dnt* and *bteA*. The *fhaB* and *prn* genes encode the filamentous hemagglutinin (FHA) and pertactin (PRN), respecitively. The FHA and PRN are important adhesins expressed in the genetic closely related species of *B. pertussis*, *B. parapertussis* and *B. bronchiseptica*, which mediates the bacterial adhesion of host cells [12, 13]. It should be aware that the PRN-deficient *B. pertussis* had emerged, and the losses of the *prn* gene expression in the strains might be driven by using of acellular vaccine containing PRN [14]. The *cyaA* gene encodes the multifunctional adenylate cyclase-hemolysin (AC-Hly) toxin, which possess adenylate cyclase activity, pore-forming activity and cell invasive activity [15]. It was showed that the AC-Hly toxin expressed in the *B. bronchiseptica* strains isolated from human and rabbit was responsible for the lethality of the intranasally infected mice [7]. The *dnt* gene encodes the dermononecrotic toxin (DNT), and the toxin is a well-recognized causative factor inducing atrophic rhinitis and bronchopneumonia in pigs [16]. Interestingly, the ability to express DNT is varied among strains. It was showed that the larger amount of DNT was produced in pig isolates than in a rabbit isolate RB50 [17]. The *bteA*, also known as *bopC*, encodes the *Bordetella* type III secretion system effector A (BteA), which is an important effector secreted from the type III secretion system [18, 19]. It was showed that BteA could induce the necrotic cell death and inhibit the macrophage phagocytosis [18, 19]. Take together, the 219 *B. bronchiseptica* isolates carrying the 5 virulence genes in this study were the potential pathogen causing severe respiratory infection in rabbits.

Antibiotics play an important role in prevention and treatment of infections caused by *B. bronchiseptica* [20–22]. However, the widespread use of antibiotics for preventing and treating the bacterial infections leads to the emergence of antibiotic-resistant strains [22–24]. It was demonstrated that *B. bronchiseptica* strains isolated from swine in Germany were resistant to ampicillin, and *B. bronchiseptica* strains isolated from companion animals in Germany and other European countries showed decreased susceptibility to β-lactam antibiotics and cephalosporines [25]. In consistence with this study, *B. bronchiseptica* isolates showing resistant to ampicillin and cephalosporines were also observed in this study. It was showed that the production of beta-lactamases and the reduced membrane permeability to cephalosporins might result in the resistance to ampicillin and cephalosporines, respectively [26]. Therefore, it should be aware that the widespread use of antibiotics to control the infection caused by *B. bronchiseptica* in rabbits is unsustainable.

Conclusion

The characteristics of the *B. bronchiseptica* strains isolated from dead rabbits with respiratory disease in Fujian Province were described in this study. The results showed that *B. bronchiseptica* was an important pathogen causing respiratory disease in rabbits in Fujian Province, and that *B. bronchiseptica* stain of ST12 that can infect humans was also isolated from rabbits in Fujian Province. The results might play important roles in tracking the epidemic strains in rabbits, controlling the *B. bronchiseptica* infections in rabbits and preventing the potential rabbit-human transmission events.

Methods

Ethics statement

Lung samples were collected from dead rabbits with respiratory disease in according with the guidelines issued by the Research Ethics Committee of the Institute of Animal Husbandry and Veterinary Medicine, Fujian Academy of Agriculture Sciences (FAAS). This study was also approved by the Research Ethics Committee of the Institute of Animal Husbandry and Veterinary Medicine, and the approved number is FAAS-AHVM2017–0511.

Sample collection and B. bronchiseptica isolation

Nine rabbit farms in Fuzhou, Longyan and Nanping cities in Fujian Province were included for sampling. The 2 rabbit farms in Fuzhou raise Fujian Yellow rabbit (local rabbit breed), the 3 rabbit farms in Longyan raise Fujian White rabbit (local rabbit breed), the other 2 rabbit farms in Longyan raise Minxinan Black rabbit (local rabbit breed), and the 2 rabbit farms in Nanping raise Hyplus rabbit (foreign rabbit breed). Lung samples were collected from dead rabbits with respiratory disease manifested with nasal discharge and/or matted forepaws. In all, 833 lung samples were collected from August 2017 to December 2019.

In order to isolate *B. bronchiseptica*, each sample was mixed with sterile phosphate buffer saline and homogenized to make 50% suspension. One hundred microliter of suspension was spread on brain heart infusion (BHI) agar plate (containing 1% glycerol and 10% defibrinated sheep blood) and incubated for 48 to 72 h at 37˚C. Five Gram-negative suspected isolates from each plate were picked up. The identities of the isolates with oxidase and ornithine decarboxylase positives, and glucose fermentation negative were further confirmed by sequencing of the 16S rRNA genes. One isolate from each sample was selected as representative for further characterization.

MLST analyses

The isolates were analyzed by MLST as described in PubMLST website (https://pubmlst.org/). Briefly, 7 house-keeping genes including *adk*, *fumC*, *glyA*, *tyrB*, *icd*, *pepA* and *pgm* were amplified and sequenced. The seven allelic numbers were given by comparing the seven house-keeping genes of the isolate to the corresponding genes in the PubMLST database. The sequence type (ST) of the isolate was defined according to the seven allelic numbers.

Virulence genes detection

Five well-characterized virulence genes that thought to be involved in interactions with the host were screened in the isolates, including filamentous hemagglutinin (*fhaB*), pertactin (*prn*), adenylate cyclase-hemolysin toxin (*cyaA*), dermononecrotic toxin (*dnt*) and the *Bordetella* type-III secretion system effector A (*bteA*). The primers for amplification of the five virulence genes were designed based on the corresponding genes in the NCBI database (https://www.ncbi.nlm.nih.gov/) (Table 3). PCR reaction mixtures comprised 25 μL 2×*EasyTaq* PCR SuperMix (TransGen Biotech, Beijing, China), 0.2 μM of each forward and reverse primer, and 100 ng bacterial genome DNA in a final volume of 50 μL. The cycling conditions for PCR assays were 94 °C for 5min and 35 cycles of 94 °C for 30 s, 59 °C for 30 s and 72 °C for 30 s, followed by a final elongation step of 72°C for 5 min. The PCR products were sequenced to confirm the identities.

Antimicrobial susceptibility test

The antimicrobial susceptibility test of the isolates were conducted by using disc diffusion method on BHI agar plate (containing 1% glycerol and 10% defibrinated sheep blood) according to the Clinical and Laboratory Standards Institute (CLSI) standards. Ten antibiotics including ampicillin, cefixime, cefotaxime, ceftizoxime, gentamycin, kanamycin, streptomycin, ofloxacin, ciprofloxacin and norfloxacin were used. The results were interpreted based on the breakpoints for *Enterobacteriaceae* of the CLSI standards [27,28]. The *Staphylococcus aureus* ATCC 29213 was included as the quality control.

Abbreviations

B. bronchiseptica
Bordetella bronchiseptica
STs
Sequence types
MLST
Multi-locus sequence typing
PCR
Polymerase chain reaction
P. multocida
Pasteurella multocida
E. coli
Escherichia coli
K. pneumoiae
Klebsiella pneumoiae
FHA
Hemagglutinin
PRN
Pertactin
AC-Hly
adenylate cyclase-hemolysin
DNT
Dermononecrotic toxin
BteA
Bordetella type III secretion system effector A
BHI
Brain heart infusion

Declarations

Ethics approval and consent to participate

This study was approved by the Research Ethics Committee of the Institute of Animal Husbandry and Veterinary Medicine, and the approved number is FAAS-AHVM2017–0511. We obtained written informed consent from the owners of the animals for using them in this study.

Consent for publication

Not applicable.

Availability of data and material

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interest.

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Authors' contributions

Conceived and designed the experiments: JXW and XPX. Performed the experiments: JXW and SKS. Sample collection: JXW, YFC and SKS. Analyzed the data: JXW, DJC and LS. Contributed to the writing: JXW and XPX. All authors have read and approved the final manuscript.

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Figures

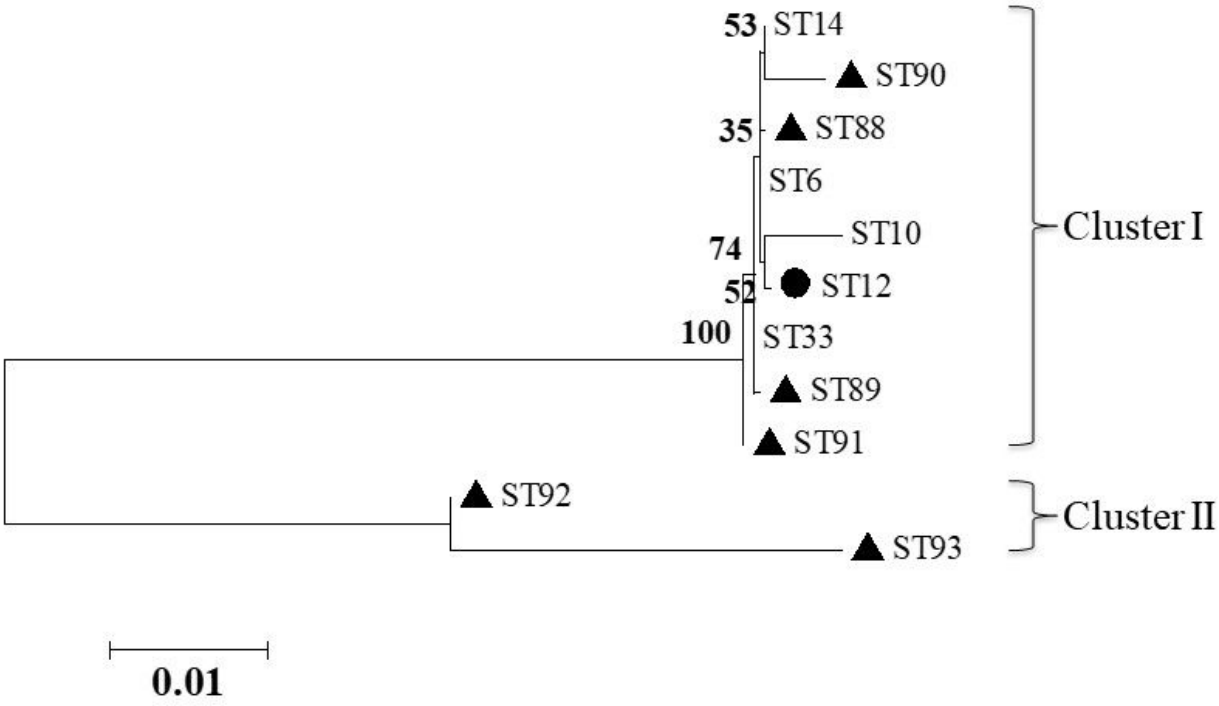

Figure 1

Phylogenetic tree of the 11 STs of B. bronchiseptica based on the concatenated 7 house-keeping genes. The black filled triangles indicate the new STs. The black filled circle indicates the B. bronchiseptica strain of ST12 that can be isolated from both rabbits and humans.