RESEARCH ARTICLE

Characterization and whole genome sequencing of a novel strain of *Bergeyella cardium* related to infective endocarditis

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Abstract

Background: *Bergeyella cardium* infection is becoming increasingly prevalent in patients with infective endocarditis, suggesting its significance in disease pathogenesis. However, few studies have fully characterized this species.

Results: Herein, we report the morphological and physiological characteristics, as well as whole genome sequencing of a newly identified *B. cardium* HPQL strain isolated from a patient with infective endocarditis. Results from the cellular morphology and biochemical analysis provide basic knowledge on the new pathogen. The whole genome sequencing of *B. cardium* HPQL consists of a circular chromosome with a total length of 2,036,890 bp. No plasmid was detected. Comparative genomics were carried out then. Antibiotics resistance related genes, pathogenesis related genes, predicted insertion sequences, genome islands and predicted CRISPRs sequences were demonstrated. To our knowledge, this is the first study to provide a complete genome sequence for *Bergeyella* spp.

Conclusions: This study provides fundamental phenotypic and genomic information for the newly identified fastidious infective endocarditis causative bacteria, *B. cardium*. Our results provide insights into effective clinical diagnosis and treatment of this pathogen.

Keywords: *Bergeyella cardium*, Infective endocarditis, Genome sequencing

Background

Infective endocarditis is a serious infectious disease with high associated morbidity and mortality. Identification of the causative agents is, therefore, crucial for improving the clinical outcome [1]. Clinically, infective endocarditis is generally diagnosed based on positive blood cultures, removed leads, and/or infected pocket material [1]. Species belonging to the genera *Streptococcus*, *Staphylococcus*, and *Enterococcus* are the primary causative organisms of infective endocarditis [2]. However, recently new pathogens are emerging as additional etiological agents, such as *Bergeyella* spp. [3–5].

*Bergeyella* spp. are non-fermenting gram-negative bacilli, belonging to the family *Flavobacteriaceae* [3]. *B. zoohelcum*, known to cause cellulitis, leg abscess, tenosynovitis, septicemia, pneumonia, and meningitis, is one of the best described zoonotic pathogens afflicting humans [6, 7]. *B. zoohelcum* is usually isolated from the normal oral microflora of animals such as cats and dogs [8]. Hence, animal bites and prolonged exposure to pets are the primary causes of human infection with *B. zoohelcum* [4]. In addition, a patient suffered *B. zoohelcum* bacteremia after eating food prepared with coagulated goat blood [9, 10]. A case of cellulitis due to *B. zoohelcum* infection was also reported in a tsunami victim [9]. Alternatively, infections caused by other *Bergeyella* spp. are rarely reported. A previously uncultivated *Bergeyella* sp. (clone AF14) with strong homology to a previously reported uncultivated oral *Bergeyella* strain was suspected to be an opportunistic pathogen during preterm birth [11]. Further, the isolation of two *Bergeyella* strains was reported from patients with infective endocarditis. Both strains shared 94.9% homology with *B. zoohelcum*, suggesting that they are a new species belonging to the genus *Bergeyella*. The two strains were designated as *Bergeyella cardium*13-07T and *Bergeyella cardium*13–16 [3]. Meanwhile, another case study reported the isolation of a *Bergeyella* strain from an infective endocarditis
patient that had 98.2% shared identity with \textit{B. zoohel-}
\textit{cum}, which was slightly lower than the ≥99.0% ho-
omology required for two organisms to be considered the
same species \cite{4}. Recently, a novel \textit{Bergeyella} sp. was
isolated from a patient with infective endocarditis. The
organism was determined to be genetically most closely
related to \textit{B. cardium} \cite{12}. Moreover, the first case of \textit{B. cardium}
prosthetic valve endocarditis was also reported
quite recently \cite{5}.

The increasing number of cases of \textit{B. cardium} infec-
tions in patients with infective endocarditis suggests its
importance in disease pathogenesis. However, studies
examining the microbial characteristics and genetic
features of this species are very rare. In this study, we
therefore, sought to describe the isolation, identification
and characterization of a new \textit{B. cardium} sp. from blood
cultures of a patient with infective endocarditis. We also
performed whole genome sequencing and, through
phylogenetic analysis, we were able to predict the pos-
sible origin of this newly identified species.

\textbf{Results}

\textbf{Phylogenetic analysis identified the isolate as a novel
species of \textit{B. cardium}}

Four days after the initial blood culture was obtained
from a 63-year-old man with infective endocarditis, the
growth of microorganisms was reported through an
automated blood culture system. Gram staining revealed
the presence of gram-negative bacilli. However, no
reliable identification was made by matrix-assisted laser
desorption/ionization-time of flight mass spectrometry
(MALDI-TOF MS). Thus, to accurately identify the
pathogenic species, a 1425 bp sequence from the 16S
rRNA gene of the isolated strain was amplified and
sequenced via polymerase chain reaction (PCR). The
sequence was then submitted to NCBI BLASTN to iden-
tify matched bacterial sequences. Results revealed that
the sequence with the highest homology (approximately
99%) was isolated from \textit{B. cardium}. To further verify the
nucleotide BLAST results, a detailed phylogenetic tree
was constructed as shown in Fig. 1. Results showed the
isolated strain clustering with two \textit{B. cardium} strains
that had been isolated from two infective endocarditis
patients in Korea \cite{3}. Hence, the phylogenetic analysis
supported the finding that the isolated strain was a novel
strain of \textit{B. cardium}. We, therefore designated the iso-
lated strain in our study as \textit{B. cardium} HPQL (identified
by Hongwei Pan from QiLu hospital).

\textbf{General microbial characteristics of \textit{B. cardium} HPQL}

Morphological, physiological and biochemical characteri-
zation of the newly isolated strain was performed. We
observed that the bacterial cells aggregate together in
blood cultures (Fig. 2a). Moreover, small colonies of
\textit{B. cardium} HPQL were observed on blood agar after
48 h of incubation at 35 °C (Fig. 2c). However, the

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{phylogenetic_tree.png}
\caption{Phylogenetic tree based on 16S rRNA gene sequences. Phylogenetic analysis was performed on \textit{B. cardium} HPQL and closely related
species based on 16S rRNA gene sequences. The HPQL strain was determined to cluster with \textit{B. cardium} spp. The phylogenetic tree was created
using the Neighbor-Joining algorithm. The branches are scaled in terms of the expected number of substitutions per site.}
\end{figure}
strain did not grow on MacConkey agar or Mueller-Hinton agar plates, indicating that this organism exhibits fastidious growth patterns. Further, individual colonies grown on blood agar appeared as non-pigmented, circular, shiny, and smooth with entire edges (Fig. 2c). Microscopic and scanning electron microscope observation revealed irregularly rod-shaped bacterial cells (Fig. 2b and d).

Biochemical characteristic of the strain were further analyzed. According to the results procured from API NH, this bacterial strain did not produce penicillinase, ornithine decarboxylase, urease, β-galactosidase, proline arylamidase or gamma glutamyl transferase. However, it was positive for lipase, alkaline phosphatase and oxidase activity, and negative for catalase activity and indole production. Additionally, acid was found to be produced from D-glucose, D-fructose, D-maltose, D-sucrose.

Antimicrobial susceptibility analysis
The E-test method was ultimately selected for AST analysis. MICs were determined following 4 days of growth on Columbia blood agar plates. The MICs for many of the selected antibiotics were quite low, with the exception of fluoroquinolones, chloramphenicol, azithromycin and gentamycin (Table 1).

Genomic features of the *B. cardium* HPQL strain
The whole genome of the newly isolated *B. cardium* HPQL strain was sequenced due to its potential clinical importance in patients with infective endocarditis. The genomic details are provided in Table 2 and Fig. 3a.

Table 1 Minimum inhibitory concentration of antimicrobial agents of *B. cardium* HPQL

| Antimicrobial agent (μg/mL) | MICs after 96 h incubation |
|-----------------------------|---------------------------|
| Penicillin                  | 0.032                     |
| Ceftriaxone                 | 0.048                     |
| Cefepime                    | 0.016                     |
| Cefotaxime                  | 0.024                     |
| Meropenem                   | 0.012                     |
| Imipenem                    | 0.032                     |
| Tigecycline                 | 0.032                     |
| Amoxicillin/Clavulafiate    | 0.016                     |
| Sulfamethoxazole            | 0.094                     |
| Levofoxacin                 | 0.5                       |
| Ciprofoxacin                | 0.5                       |
| Chloramphenicol             | 3                         |
| Azithromycin                | 8                         |
| Gentamycin                  | 64                        |
Briefly, *B. cardium* HPQL contained a circular chromosome with a total length of 2,036,890 bp. The GC-content of the chromosome was determined to be 39.63%. The whole genome sequence contained 1,896 predicted coding sequences (CDS), including 9 rRNAs, 42 tRNAs and 1 sRNA. A total of 1,813,065 predicted coding sequences were identified, which occupied 89.01% of the whole genome sequence. Additionally, 70.25% (1,332/1896) of the protein-coding genes were assigned putative functions in the COG database, while the remaining genes were annotated as encoding hypothetical proteins. The distribution of genes in COGs functional categories are presented in Table 3. In addition, the methylation data of the whole genome are listed in Additional file 3 (Sheet S1), Fig. 3b and deposited to REBASE database.

**Pathogenic analysis of *B. cardium* HPQL**

A whole genome BLAST search was performed against the CARD, VFDB, and PHI databases to identify genes related to antibiotic resistance and virulence factors in the genome of *B. cardium* HPQL. Twelve genes were identified homology to well-known antimicrobial resistance genes (Additional file 4 Sheet S2). Moreover, a total of 70 genes related to putative virulence factors were identified in the genome of *B. cardium* HPQL (Additional file 5 Sheet S3), while 92 genes were described that may participate in bacteria-host interactions (Additional file 6 Sheet S4).

**Comparative genomic analysis of *B. cardium***

Three genome islands were predicted from the whole genome sequences (Additional file 7 Sheet S5). No prophage was predicted from the whole genome sequence of *B. cardium* HPQL, while 3 CRISPERs sequences (Additional file 1 Table S1), 30 insertion sequences...
Table 3 The genes of B. cardium HPQL genome in COG functional categories

| Functional class | Class_description | Gene_number |
|------------------|-------------------|-------------|
| C                | Energy production and conversion | 87          |
| D                | Cell cycle control, cell division, chromosome partitioning | 25          |
| E                | Amino acid transport and metabolism | 89          |
| F                | Nucleotide transport and metabolism | 52          |
| G                | Carbohydrate transport and metabolism | 46          |
| H                | Coenzyme transport and metabolism | 87          |
| I                | Lipid transport and metabolism | 56          |
| J                | Translation, ribosomal structure and biogenesis | 166         |
| K                | Transcription | 51          |
| L                | Replication, recombination and repair | 77          |
| M                | Cell wall/membrane/envelope biogenesis | 148         |
| N                | Cell motility | 12          |
| O                | Posttranslational modification, protein turnover, chaperones | 82          |
| P                | Inorganic ion transport and metabolism | 63          |
| Q                | Secondary metabolites biosynthesis, transport and catabolism | 19          |
| R                | General function prediction only | 98          |
| S                | Function unknown | 62          |
| T                | Signal transduction mechanisms | 37          |
| U                | Intracellular trafficking, secretion, and vesicular transport | 23          |
| V                | Defense mechanisms | 44          |
| X                | Mobilome: prophages, transposons | 8           |

(Additional file 2 Table S2) and four toxin-antitoxin (Additional file 8 Sheet S6) were predicted from the whole genome sequence of B. cardium HPQL. Moreover, comparative genomic analysis between the B. cardium HPQL, B. cardium (downloaded from NCBI PRJNA490389), B. zoohelcum ATCC 43767, B. zoohelcum CCUG 30536, B. zoohelcum NCTC 11660 and B. zoohelcum NCTC 11661 genome also demonstrated the evolutionary divergence of B. cardium HPQL from B. Zoohelcum spp. (Fig. 4). The relatively low sequence homology observed for the newly isolated B. cardium sp. with B. zoohelcum implied that the HPQL strain is a new member of the Bergeyella genus. Further comparative genomic analysis of the two B. cardium strains revealed 259 genes specific to B. cardium HPQL and 80 genes specific to another B. cardium strain.

Original analysis of the B. cardium related to infective endocarditis

To further elucidate the possible origin of the new B. cardium sp. strain, 16S rRNA sequences of the Bergeyella spp. were downloaded from NCBI for phylogenetic analysis. The analysis results demonstrated that strains homologous to B. zoohelcum clustered into one group, while strains homologous to the B. cardium strain clustered into another (Fig. 5). Interestingly, strains homologous to B. zoohelcum were isolated from animals, while the strains homologous to B. cardium were isolated from Homo sapiens. Moreover, two uncultured oral bacterial clones were identified with strong similarity to B. cardium sp., indicating that these strains also belong to the B. cardium sp.

Discussion

Little is known about the genus Bergeyella with B. zoohelcum being the only well described zoonotic pathogen currently afflicting humans [6, 7]. Recently, worldwide, 4 cases of B. cardium sp. have been reported as being isolated from patients with infective endocarditis [3, 5, 12]. The isolates were recognized as a novel strains belonging to the genus Bergeyella. In this study, we reported an additional new isolate belonging to the Bergeyella genus, from blood cultures of infective endocarditis patients. Results from NCBI BLASTN and phylogenetic analyses reveal that the new isolate belonged to B. cardium (Fig. 1). The 4 previously reported cases together with our new discovery clearly suggest that B. cardium sp. is correlative with human infective endocarditis. However, few studies have examined the fundamental biological properties of these new strains. Herein, we provide detailed biological characterization and whole genome sequencing of the newly isolated B. cardium sp. Our study may, therefore, serve to provide fundamental information to better understand this newly identified pathogen.

Consistent with other four reported cases, our isolate was also fastidious and was found to grow slowly on blood agar. The fastidious nature of the B. cardium sp. may account for their rare isolation. In addition, the newly identified strain exhibited irregular rod-shaped cells similar to B. cardium PU13217 [12]. Biochemical analysis using API card was also carried out, which may provide better understanding of this new strain of Bergeyella. Furthermore, our AST results were consistent with that observed for strain 13-7 T, demonstrating similar MIC susceptibilities in response to antimicrobial agents (Table 1). Currently there are no clearly defined standards described by NCCLS/CLSI for antibiotic susceptibility testing or breakpoints for B. cardium; however, our AST data, together with previous studies [3, 12], suggest effective targeted antibiotics for treatment of infections with this bacterial species.

We also sequenced the complete genome of B. cardium strain HPQL. To our knowledge, this is the first complete genome sequencing performed on any
Bergeyella spp. Analysis results revealed 12 genes related to antibiotic resistance (Additional file 4 Sheet S2), including 3 that related to fluoroquinolone resistance, which is consistent with our in vitro AST analysis results. Sequencing results also revealed 162 genes associated with encoding virulence factors (Additional file 5 Sheet S3 and Additional file 6 Sheet S4). Identification of these genes serve to the current understanding of the mechanisms responsible for the pathogenic effects elicited by B. cardium strains.

The B. cardium strains isolated from this human patient were phylogenetically unique compared to the strains isolated from animals (Fig. 5), suggesting that the newly identified strains may originate from different sources than those that infect animals.

**Fig. 4** Comparative genomic analysis. Comparative genomic analysis between the B. cardium HPQL, B. cardium (downloaded from NCBI PRJNA490389), B. zoohelcum ATCC 43767, B. zoohelcum CCUG 30536, B. zoohelcum NCTC 11660 and B. zoohelcum NCTC 11661 genomes was carried out. Phylogenetic tree based on core genome analysis.

**Fig. 5** Phylogenetic tree based on 16S rDNA sequences from multiple Bergeyella spp. Phylogenetic analysis of Bergeyella zoohelcum (Group A, animal original) and (Group B, human original) homologous to Bergeyella cardium strain. The phylogenetic tree was created using the Neighbor-Joining algorithm. The branches are scaled in terms of the expected number of substitutions per site.
Conclusions
Our data, collectively with other studies, clearly document that B. cardium strains are important, newly identified, human pathogens. The phylogenetic, phenotypic and morphological results together with the whole genome sequencing serve to extensively expand the current knowledge on the newly identified Bergeyella spp. as it relates to human infective endocarditis. Furthermore, our results provide insights into effective clinical diagnosis and treatment of this pathogen. We also suggest that this specific strain of B. cardium originated from the human oral cavity, though direct evidence of this was lacking. Future studies should focus on elucidating the pathogenic mechanisms elicited by this newly identified pathogen.

Methods
Bacteria isolation
A 63-year-old male presented to Qilu Hospital at Shandong University, Jinan, China, on April 26, 2016 with intermittent fever, fatigue, and chest distress for the previous 10 months. Ultrasonic cardiogram revealed infective endocarditis with valvular disease. Blood samples were sent to the microbiology laboratory for culturing on April 30, 2016. The blood cultures were incubated in the Bactec system (Becton Dickinson, Franklin Lakes, NJ) until a positive result was obtained. The positive blood cultures were inoculated onto Columbia blood agar, MacConkey agar, and Chocolate agar and incubated (Thermo Fisher Scientific, USA) at 35 °C until visible colonies appeared. Colonies were purified using blood agar for further analysis.

Phylogenetic analysis
The nucleotide sequences of 16S rRNA genes from different bacterial strains were downloaded from the NCBI database (http://www.ncbi.nlm.nih.gov) and aligned using the ClustalX computer program. The aligned sequences were refined and phylogenetically analyzed using distance/neighbor joining (NJ) and maximum-likelihood (ML) algorithms with the Poisson correction distance model in the MEGA software package [13] to infer their phylogenetic relationships. The bootstrapping supports for the interior branch length of the trees were from 1000 replicates.

Morphological, physiological and biochemical characterization
Morphological characterization of the isolated bacterial strain was carried out as previously described [14]. Growth was examined on Columbia blood, MacConkey and Chocolate agar. The strain was further biochemically characterized using API NH card (bioMérieux, Marcy l’Étoile, France) according to the manufacturer’s instructions.

Antibiotic sensitivity analysis
Both the Vitek 2 system (bioMérieux, Marcy l’Étoile, France) and PDM Epsilometer test (E test) were employed to determine the antibiotic susceptibility of the isolated strain. For the Vitek 2 system, the cell density of the bacterial colony was adjusted to a density of 0.5 McFarland with 0.45% saline; 145 μL of the bacterial suspension was then added into 3 mL of 0.45% saline solution to further adjust the bacterial cell density. The suspension vials were then applied to the Vitek GN09 card and loaded into the Vitek 2 automated reader-incubator for analysis. For the E test, the 0.5 McFarland bacterial cell suspension were surface plated onto Blood agar plates, using a sterile swab to produce an even inoculum [15]. The plates were then incubated for 96 h (Thermo Fisher Scientific, USA) at 35 °C. The minimum inhibitory concentration (MIC) was determined to be the point where the elliptical zone of growth inhibition intersected with the MIC scale on the E test strip [15]. Sensitivities to penicillin, ceftriaxone, cepafime, cefotaxime, meropenem, imipenem, tigecycline, amoxicillin/clavulanate potassium, sulfamethoxazole, levofloxacin, ciprofloxacin, chloramphenicol, azithromycin, and gentamycin were examined.

Genome sequencing and assembly
Genomic sequencing and assembly were carried out at Novogen Bioinformatics Technology Co., Ltd. (Beijing, China). Single-molecule real-time (SMRT®) sequencing was performed using a Pacific Biosciences RSII sequencer (PacBio, Menlo Park, CA) according to the manufacturer’s instructions (MagBead Standard Seq v2 loading, 1 × 180 min movie) using P4-C2 chemistry. The low-quality reads were filtered by the SMRT 2.3.0 and the filtered reads were then assembled to generate one contig without gaps. Hierarchical Genome Assembly Process (HGAP) pipeline was used for the whole genome assemble.

Genome annotations
The assembled genome sequence was annotated further. Small RNAs (sRNAs) were predicted by BLAST against the Rfam [16] database. tRNAscan-SE [17] was then used to predicted transfer RNA (tRNA) genes, while the rRNAmer server [18] was used to predict ribosomal RNA (rRNA) genes. RepeatMasker [19] and Tandem Repeat Finder [20] were applied to predict repetitive sequences and tandem repeats, respectively. A whole genome alignment (E-value less than 1e-5 and a minimal alignment length percentage > 40%) against 6 databases, namely Clusters of Orthologous Groups (COG), Kyoto Encyclopedia of Genes and Genomes (KEGG), NCBI non-redundant (NR), Swiss-Prot, Gene Ontology (GO) and Translated EMBL (TrEMBL) was performed to predict gene functions [21–27]. ISFinder blast (https://www-is.
of bootstraps was 1,000 with the orthologous genes.
eST [35] according to the PhyML method, and the set-
phylogenetic tree was also constructed using the TreeB-
cutoff in length difference of amino acids [25, 33, 34]. A
with the threshold set to 50% pairwise identity and a 0.7
CD-HIT rapid clustering of similar proteins software
NCBI). Core genes and specific genes were analyzed via
and
B. zoohelcum
NCTC 11660 genome (downloaded from NCBI),
CCUG 30536 genome (downloaded from NCBI),
B. zoo-
genome (downloaded from NCBI), the
B. zoohelcum
CP029149.

Additional file 2: Table S2. The predicted CRISPR sequences in the
genome of B. cardium HPQL.

Additional file 3: The methylation data of the whole genome.

Additional file 4: The predicted genes related to antibiotic resistance.

Additional file 5: Genes related to putative virulence factors.

Additional file 6: Genes related to bacteria-host interactions.

Additional file 7: The predicted genome islands

Additional file 8: The predicted toxin-antitoxins

Prediction of genes related to antibiotic resistance and
virulence factors
The genome sequences of the HPQL bacterial strain were
submitted to the Virulence Factors of Pathogenic Bacteria (VFD)
[30], Comprehensive Antimicrobial Resistant Database (CARD)
[31] and Pathogen-Host Interactions database (PHI) [32] databases to predict which genes were
related to antibiotic resistance and virulence factors.

Comparative genomics analysis
Comparative genomic analysis was performed between the
B. cardium HPQL genome, B. cardium (downloaded from
NCBI PRJNA490389) B. zoohelcum ATCC 43767
(genome (downloaded from NCBI), the B. zoohelcum
CCUG 30536 genome (downloaded from NCBI), B. zo-
helcum NCTC 11660 genome (downloaded from NCBI),
and B. zoohelcum NCTC 11661 (downloaded from NCBI).
Core genes and specific genes were analyzed via
CD-HIT rapid clustering of similar proteins software
with the threshold set to 50% pairwise identity and a 0.7
cutoff in length difference of amino acids [25, 33, 34]. A
phylogenetic tree was also constructed using the TreeB-
eST [35] according to the PhyML method, and the setting of bootstraps was 1,000 with the orthologous genes.

Nucleotide sequence accession numbers
The obtained genome sequence for B. cardium HPQL
was deposited in GenBank under the accession numbers CP029149.

Supplementary information
Supplementary information accompanies this paper at https://doi.org/10.1186/s12866-020-1715-0.

Additional file 1: Table S1. The predicted CRISPR sequences in the
genome of B. cardium HPQL.

Abbreviations
B. cardium: Bergeyella cardium; CARD: Comprehensive Antimicrobial Resistant Database; CDS: Coding sequences; COG: Clusters of Orthologous Group; ESR: Erythrocyte sedimentation rate; GO: Gene Ontology; HPQL: Hongwei Pan from QiLu hospital; KEGG: Kyoto Encyclopedia of Genes and Genomes; MIC: Minimum inhibitory concentration; ML: Maximum likelihood; NJ: Neighbor joining; NR: NCBI non-redundant database; PHI: Pathogen-Host Interactions database; RASTEmbl: Translated EMBL; VFDB: Virulence Factors of
Pathogenic Bacteria

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Authors’ contributions
YZ, ES and HP conceived and designed the experiments; HP and LW, performed the experiments; HP, YZ, and ES wrote the paper. All authors read and approved the final manuscript.

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collection and interpretation, or the decision to submit the work for publication.

Availability of data and materials
The obtained genome sequence for B. cardium HPQL was deposited in GenBank under the accession numbers CP029149. In addition, the
methylation data of the whole genome, whole genome sequences and
predicted restriction modification system were also available in REBASE database (http://rebase.neb.com/rebase/private/pacbio_Pan15.html). All data
generated or analysed during this study are included in this published article and its supplementary information files. The datasets used and/or analysed during the current study are also available from the corresponding author on reasonable request.

Ethics approval and consent to participate
This study was approved by the ethics committee of QiLu Hospital,
Shandong University, Jinan, People’s Republic of China (protocol KYLL-2019
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