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Antimicrobial resistance and virulence factors analysis of a multidrug-resistant *Acinetobacter baumannii* isolated from chickens using whole-genome sequencing

Lumin Yu[1*], Yuzhong Zhao[1], Shanpeng Zhang[1], Weishi Ni[1], Lingling Zhang[1], Cong Xue[1], Peikun Wang[1] and Xinglin Zhang[1*]

Abstract

Multidrug-resistant (MDR) *Acinetobacter baumannii* (*A. baumannii*) is currently recognized not only as a significant nosocomial pathogen but also is an emerging bacterial infection in food-producing animals, posing a critical threat to global health. However, this is a hindrance to detailed bioinformatic studies of MDR *A. baumannii* of chicken origin due to the lack of its complete genome sequence. Here, we report whole-genome sequencing analysis of MDR *A. baumannii* Y03 isolated from chickens. The Y03 genome consists of 1 circular chromosome and 4 circular plasmids, The Y03 chromosome harbors 41 antimicrobial resistance genes conferring resistance to major classes of antibiotics, including β-lactams, phenicols, macrolides, lincosamides, aminoglycosides, and nitrofurans, as well as 135 virulence factors involved in effector delivery system, immune modulation, adherence, stress survival, biofilm, exotoxin, and nutritional/metabolic factor. The in vivo infection experiments certificated that Y03 was virulent to chickens. Meanwhile, we used PCR amplification method to detect 10 antimicrobial resistance genes including *abeM*, *adeB*, *adeH*, *adeK*, *blaapmC*, *blaOXA−90*, *catB9*, *macB*, *folP*, and *parE*, as well as 14 virulence genes including *lpxC*, *pilO*, *fimT*, *ompA*, *basA*, *bauA*, *gspL*, *csu*, *pgaC*, *plc2*, *tssA*, *tviB*, *bap*, and *vgrG*. Whole-genome sequencing analysis revealed that Y03 contained 46 horizontal gene transfer elements, including 11 genomic islands, 30 transposons, and 5 prophages, as well as 518 mutations associated with reduced virulence and 44 mutations resulting in loss of pathogenicity. Furthermore, there were 22 antibiotic targets and 28 lethal mutations on the Y03 chromosome that could be used as potential targets to prevent, control, and treat infections caused by MDR *A. baumannii* Y03. Therefore, this study contributes to the development of strategies for the prevention, control, and treatment of *A. baumannii* infections and their spread in chickens.

Keywords Multidrug-resistant *Acinetobacter baumannii*, Whole-genome sequencing, Antimicrobial resistance, Virulence factors

*Correspondence:
Lumin Yu
yulumin@lyu.edu.cn
Xinglin Zhang
zhangxinglin@lyu.edu.cn
[1]College of Agriculture and Forestry, Linyi University, Linyi, Shandong 276005, China

Introduction

Acinetobacter baumannii (*A. baumannii*) is a strictly aerobic, non-fermenting, Gram-negative coccobacillus with pili and capsule, but no flagella [1–3]. It has emerged as an important opportunistic pathogen of nosocomial infections responsible for high mortality and morbidity rates during the last decades due to its ability for survival in the hospital environment on a wide range of dry and moist surfaces [1, 4–6]. *A. baumannii* could cause various hospital-acquired infections, including pneumonia, endocarditis, bacteremia, urinary tract infections, meningitis, peritonitis, skin and wound infections, particularly among severely ill patients in the intensive care unit (ICU) and immunocompromised individuals [3, 4, 6–8]. These infections are closely connected with the implantation of the ventilators, vascular catheters, and cerebrospinal fluid shunts, on which *A. baumannii* colonizes and adheres [5, 9]. Therefore, *A. baumannii* has drawn the attention of medical professionals worldwide as a public health threat.

The ability of *A. baumannii* to survive in the hospital environment and inside the host for extended periods is due to its possession of a series of virulence factors, including capsular polysaccharides, lipopolysaccharides, type I fimbriae, P fimbriae, curli fiber, chaperone-usher type I pili, type IV pili, invasins, biofilm-associated protein, outer membrane proteins, outer membrane vesicles, serum resistance, phospholipases, acinetobactin, fimsbactin, baumannoferrin, etc [5, 10–16]. These virulence factors are categorized into four main groups: adhesion and invasion, toxins, protein secretion systems, and iron acquisition, which facilitate *A. baumannii* to adhere and colonize host tissues and organs, disseminate, invade host cells, and evade the host immune response [16–18].

Antibiotics nowadays are used to prevent, control and treat *A. baumannii* infections and outbreaks [1, 19]. However, the continued overuse and misuse of antibiotics have enabled *A. baumannii* to develop different types of resistance mechanisms, e.g., the production of degradative enzymes, a change in metabolic status, a decrease in bacterial membrane permeability, the alteration of antibiotic targets, the overexpression of efflux pumps, and the formation of biofilms [5, 10, 12, 20, 21]. Documented data revealed that the *A. baumannii* isolates harbored the high prevalence of antibiotic resistance against a wide range of clinically effective antibiotics [3, 5]. Accordingly, they are classified into three categories: multidrug-resistant (MDR) strains, extensively drug-resistant (XDR) strains, and even pan-drug-resistant (PDR) strains, representing a significant challenge for therapy in clinics [3, 11, 16, 20].

Whole-genome sequencing provides an unprecedented level of information on species identification, antimicrobial resistance, and the molecular epidemiological typing of microorganisms to researchers, and it also helps in the identification of new drug targets and the development of new drugs [16, 22–25]. More studies about the pathogenicity, the prevalence and antibiotic resistance of *A. baumannii* isolated from human clinical isolates have been reported [2, 3, 7]. However, studies on their pathogenicity, antibiotic resistance and genomes in food-producing animals are somewhat limited. The presence of *A. baumannii* in food-producing animals is currently considered a major public health problem since it could be disseminated to community and hospital settings through the food chain [2]. Chickens are the most widely kept food-producing animals in the world due to their abilities to providing high quality protein and income for the rural households [26]. Upon infection of chickens with *A. baumannii*, the bacteria could be transmitted directly from chickens and their products (i.e., meat and eggs) to farm workers and consumers, and indirectly to a larger population through contaminated food, water, and soil [26].

The aim of this study was to investigate the whole genome, pathogenicity and antibiotic resistance of *A. baumannii*, and to understand the pathogenicity and antibiotic resistance mechanisms of *A. baumannii* in chickens and the differences between it and *A. baumannii* isolates from other sources. Therefore, this study contributes to the development of strategies for the prevention, control and treatment of *A. baumannii* infections and their spread in chickens.

Materials and methods

Isolation and identification of *A. Baumannii*

A bacterial disease broke out in a chicken farm in Linyi, Shandong province, China. The diseased chickens presented obvious clinical symptoms of listlessness, tachypnea, anorexia, runny nose, hypothermia, and lethargy with closed eyes. For the isolation of bacteria, the chickens were euthanized by injecting intravenous sodium pentobarbital (100 mg/kg) into the wing vein and dissected. The pathological changes of the chicken autopsy showed slight bleeding in the lungs, mottled bleeding in the livers and heart, tumefaction in the spleen and kidneys. And then approximately 5 g of heart, livers, spleen, lungs, and kidneys were collected and homogenized in a stomacher (Scientz, Ningbo, Zhejiang, China) for 2 min, respectively. Each sample was added in 10 mL of the sterile phosphate-buffered saline (PBS) buffer (pH 7.4) and carried out 10-fold serial dilutions through 4 microfuge tubes containing 900 μL of PBS. 100 μL of the above each dilutions were then dropped and spread onto *Acinetobacter* chromogenic media (CHROMagar, Paris, France), incubated at 37 °C for 16–24 h, and examined for the growth of typical red colonies of the genus *Acinetobacter*. Subsequently, one typical colony morphological

representative of the genus *Acinetobacter* was inoculated on Luria-Bertani (LB) agar plates and further confirmed by microbiological and biochemical tests such as gram staining, oxidase, catalase, urea urease, simon citrate, MR-VP, motility and indole production, and PCR amplification using the specific primer *parC*-F/R of *A. baumannii*. The confirmed *A. baumannii* was tentatively named Y03 and stored in LB broth supplemented with 25% sterile glycerol at -80 °C. Before performing each assay, pure culture of *A. baumannii* Y03 was accomplished by inoculation on a LB agar plate and incubated overnight at 37 °C.

Whole-genome sequencing and library construction

Genomic DNA of Y03 was extracted using the TIANamp Bacteria DNA kit (Tiangen, Beijing, China) according to the manufacturer's instructions. The quality, purity, integrality, and yield of the extracted genomic DNA were detected using 0.35% agarose gel electrophoresis and a NanoDrop2000 spectrophotometer (Thermo Fisher, Pittsburg, PA, USA), and quantified using a Qubit 4.0 fluorometer (Invitrogen, Carlsbad, CA, USA). Whole-genome sequencing was performed on the Illumina NovaSeq platform and the Oxford Nanopore ONT platform. Libraries were constructed for sequencing using TruSeqTM DNA Sample Prep Kit (Illumina, San Diego, CA, USA) for Illumina TruSeq Nano DNA LT, and quantified using a Qubit 4.0 fluorometer (Invitrogen). All of the above processes were performed at Shanghai Personal Biotechnology Co. Ltd (Shanghai, China). After the completion of the genome assembly, the whole-genome sequence of Y03 was compared with that of *A. baumannii* published using the NCBI Genbank nucleotide sequence database (https://www.ncbi.nlm.nih.gov/).

Genome functional component analysis

Genome functional component analysis of Y03 was performed to predict the protein coding genes, non-coding RNA (ncRNA), clustered regularly interspaced short palindromic repeats (CRISPRs), and the repetitive sequences. The protein coding genes were predicted using GeneMarkS software (http://topaz.gatech.edu/GeneMark/). ncRNA includes small RNA (sRNA), ribosome RNA (rRNA), transfer RNA (tRNA), small nuclear RNA (snRNA), small nucleolar RNA (snoRNA), and microRNA (miRNA). tRNA was predicted using tRNAscan-SE software (http://lowelab.ucsc.edu/tRNAscan-SE/), and rRNA was predicted using Barrnap software (http://www.vicbioinformatics.com/software.barrnap.shtml). The perdiction of the other ncRNA was obtained by comparison with the Rfam database (http://rfam.xfam.org/). CRISPRs was predicted using CRISPR finder (http://crispr.i2bc.paris-saclay.fr/Server/). The interspersed repeats were predicted by comparison with the Repbase database (https://www.girinst.org/repbase/).

Genome subsystem analysis

Genome subsystem analysis of Y03 was performed to predict prophage, genomic islands (GIs), virulence factors of pathogenic bacteria (VFDB, http://www.mgc.ac.cn/VFs/main.htm), the comprehensive antibiotic resistance database (CARD, https://card.mcmaster.ca/), and the carbohydrate-active enzymes database (CAZy, http://www.cazy.org/). The prophage on the genome was predicted using the PHASTER tool (http://phaster.ca/), GIs using the IslandViewer 4 database (http://www.pathogenomics.sfu.ca/islandviewer/), VFDB and CARD using the BLAST tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi), and CAZy using the hmmscan software (http://hmmer.org/).

Genome functional annotation analysis

The various databases were utilized to analyze the protein coding gene functions of Y03, including the non-redundant protein database databases (NR, https://ftp.ncbi.nih.gov/blast/db/), the clusters of orthologous groups (COG, http://eggnogdb.embl.de/#/app/home/), the kyoto encyclopedia of genes and genomes (KEGG, http://www.genome.jp/kegg/), the gene ontology (GO, http://www.geneontology.org/), the transporter classification database (TCDB, http://www.tcdb.org/), and the pathogen host interactions (PHI, http://www.phi-base.org/). Besides, the secretory proteins were also predicted using the SignalP tool (http://www.cbs.dtu.dk/services/SignalP/) and the TMHMM tool (http://www.cbs.dtu.dk/services/TMHMM/).

Antimicrobial susceptibility testing

Antimicrobial susceptibility profile of Y03 was assessed by the Kirby-Bauer (KB) disk diffusion method according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2021) using the Mueller-Hinton (MH) agar (Haibo, Qingdao, Shandong, China). The antimicrobial disks included ampicillin (10 μg), aztreonam (30 μg), penicillin G (10 μg), piperacillin (100 μg), oxacillin (1 μg), cefalotin (30 μg), cefazolin (30 μg), cefotaxime (30 μg), cefoxitin (30 μg), cefuroxime (30 μg), ceftazidime (30 μg), cefoperazone (75 μg), cefepime (30 μg), cotrimoxazole (23.75/1.25 μg), chloramphenicol (30 μg), florfenicol (30 μg), clarithromycin (15 μg), erythromycin (15 μg), midecamycin (30 μg), clindamycin (2 μg), ciprofloxacin (5 μg), levofloxacin (5 μg), ofloxacin (5 μg), norfloxacin (10 μg), tetracycline (30 μg), minocycline (30 μg), amikacin (30±7.5 μg), tobramycin (10 μg), streptomycin (10 μg), gentamicin (10±2.5 μg), kanamycin (30 μg), spectinomycin (100 μg), nitrofurantoin (300 μg),

Table 1 Oligonucleotide primers used in this study

Primer name	Oligonucleotide sequence (5′-3′)	Product size/bp
parC-F	TCGCGGAAAGCTCATCTTGT	410
parC-R	TGATGCGGAAGCAGTGATGA	
abeM-F	GAATGTCACGTCGTTTCGGT	1011
abeM-R	CGGCCTAGAGCAATAAAAGA	
adeB-F	GCACGTTTTCCAAGTGTGGC	1604
adeB-R	CCCTGATCTTCCTCTGGCAT	
adeH-f	CATCAAAACAAAACTGGTTG	1092
adeH-R	CATCAAATAAAGGTAACGAC	
adeK-F	TGCAAAAAGTATGGTCTATTTCAGG	1110
adeK-R	CAGACAATGCAATTTTCTGATCAGT	
blaapmC-F	TTTTAGTACCTCAATTTATGCGGAC	864
blaapmC-R	AACGTTGCCGGATAAGAAAA	
blaOXA−90-F	ACAAGCGCTATTTTTATTTCAGCCT	634
blaOXA−90-R	CCCAACCACTTTTTGCGTATATTTT	
catB9-F	CCTCTAATTAACACACCAGT	489
catB9-R	CTGAGGAAAACGGTATTTAA	
folP-F	TACCAAAGCAGATTTTACAG	643
folP-R	GGATAACCAAGTTCATTAAG	
macB-F	TAACTCGCTTGCGACAAGAG	1048
macB-R	ACATCAAGGTCAATTGGAGC	
parE-F	GTGACACAATATACGGCTCA	1413
parE-R	GTCTACACCAATTGCAATCG	
vgrG-F	TCTTACAACGTATAGAAGGC	2077
vgrG-R	ATGTCTTGAATAAAGCCACC	
lpxC-F	ATGGTGAAACAGCGTACTCT	681
lpxC-R	ACCTTCTTCGTTCACCACAC	
pilO-F	ATGAACAACTACGGTAGTTG	560
pilO-R	CCTACGTATCGGTATGTTTT	
fimT-F	TGAGCTCACTATAACACTCG	400
fimT-R	GATAAAACTGCCATTCGACT	
ompA-F	CACTTGCTACTATGCTTGTT	803
ompA-R	ACCAGTGTTATCTGTGTGAC	
basA-F	TTTGAGTCAAAATTTAGGGC	1405
basA-R	GCTATTTAGGCGAGTAGTTT	
bauA-F	AAGGTCTTTCAATGAACAAC	1728
bauA-R	AATCAACCTTCAAACCAAGT	
gspL-F	TTTGGCATTGGTCTAACGGA	868
gspL-R	TGCTGGCTCATTCTTAAATG	
csu-F	CATTCAGAAATCACTTCTTG	402
csu-R	TGTGGTTGGTTTACAACATA	
pgaC-F	TTGCCTTTTTATATCCGTTG	936
pgaC-R	AATCCACAAACAATCCCACA	
plc2-F	GCTGCAGCGTTAGCGGCTTT	1685
plc2-R	GCTGCTTGCGTACCCGTATT	
tssA-F	TTTCTGAACTACTTAAACCC	821
tssA-R	GATTTGTACAGGCACTTGAT	
tviB-F	AATAGCAATTATTGGGTTGG	956
tviB-R	TTTCTTTAAAGCTAAGGCCC	
bap-F	TCAGTGTGGCTGATAATACT	2228
bap-R	CCAATTCATATCCACAACTT	

and polymyxin B (300 μg) in antimicrobial susceptibility determination test.

Animals

One-day-old chicks were purchased from Rizhao Langya Chicken Co. Ltd. These chicks were adequately fed food and water (a complete diet without antibiotics) and a 12 h illumination period per day. Healthy 7-day-old chicks were selected for the animal infection experiments. Euthanasia of all chicks was performed by intravenous injection of pentobarbital sodium in wing vein at a dose three times higher than the anesthetic dose. The loss of consciousness was rapid, followed by cessation of respiration and heartbeat, and exsanguination, confirming euthanasia. All procedures were carried out in accordance with the guidelines of the NIH Guide for the Care and Use of Laboratory Animals, as well as the regulations of the American Veterinary Medical Association (AVMA) regarding euthanasia, and they were approved by the Committee of Linyi University (Approval No. LYU20240206).

Animal infection experiments

After 7 days of feeding, healthy chicks were selected for the animal infection experiments to evaluate the virulence of Y03. They were divided randomly into 6 groups (group A, B, C, D, E, and F), with 8 chicks in each group. Firstly, the overnight cultures of Y03 were scraped down from LB agar plates, washed three times and resuspended in PBS, and adjusted to 4.0×10^{10}, 2.0×10^{10}, 2.0×10^{9}, 2.0×10^{8}, and 2.0×10^{7} CFU/mL, respectively. Next, chicks in group A, B, C, D, and E, were intramuscularly injected with 0.5 mL of 4.0×10^{10}, 2.0×10^{10}, 2.0×10^{9}, 2.0×10^{8}, and 2.0×10^{7} CFU/mL of Y03, respectively. The group F was intramuscularly injected with 0.5 mL of PBS, which was the negative control. The clinical signs of infected chicks, such as lethargy, anorexia and hypothermia, were observed, and the survival and death of the chicks were recorded until 7 days post-infection. The survival curve was drawn to evaluate the virulence of Y03. After 7 days, all chicks were euthanized by injecting intravenous sodium pentobarbital (100 mg/kg) into the wing vein. Chicks in group E and F were dissected and collected their heart, liver, spleen, lung, and kidney, and these organs were fixed in 4% paraformaldehyde for histological observation.

Detection of virulence genes and antimicrobial resistance genes

We used genomic DNA of Y03 as template to amplify virulence genes and antibiotic resistance genes by PCR using the corresponding primers. The sequences of primers used in this study were listed in Table 1. PCR products were electrophoresed using 1.0% agarose gel

(Sangon, Shanghai, China) containing DNA Green Stain (Vazyme, Nanjing, Jiangsu, China) and were observed by gel documentation system (Clinx, Shanghai, China).

Statistical analysis

The IBM SPSS statistics 25.0 (IBM, Armonk, New York, USA) and GraphPad Prism 8.0.1 (GraphPad, San Diego, California, USA) were used for statistical analysis. The test results were shown as means ± SDs.

Results

Identification of *A. Baumannii*

The colony morphology of this one isolate was white, circular, moist, smooth, opaque colonies on LB agar plates (Fig. 1A). Gram staining showed that this one isolate was a red coccobacillus under a 100-fold oil lens of microscope, determining that it was a Gram-negative coccobacillus (Fig. 1B). The biochemical tests showed that it was oxidase-negative, indole-negative, citratee-negative, MR, and VP-negative, catalase-positive, urease-positive, and absence of motility (data not shown). As shown in Fig. 1C and Figure S1, the gel electrophoresis band was bright, with the PCR product size around 410 bp by PCR amplification using the specific primer *parC*-F/R of *A. baumannii*. These results confirmed that this one isolate was an *A. baumannii*, which was known as Y03.

Quality assessment of whole-genome sequence assembly

We found that Y03 owned 1 chromosome and 4 plasmids by assessing the quality of whole-genome sequence assembly. As shown in Fig. 2A–E; Table 2, the Y03 chromosome consisted of 3 917 464 bp in length with a GC content of 38.92%; the 4 plasmids included 87 240 bp of plasmid 1 in length with a GC content of 34.01%, 9 243 bp of plasmid 2 in length with a GC content of 34.70%, 8 638 bp plasmid 3 in length with a GC content of 31.86%, and 534 bp of plasmid 4 in length with a GC content of 37.21%. Additionally, the genomic comparative analysis showed that the identity of the Y03 genome sequence was greater than 98% when compared to the genome sequence of *A. baumannii* published in the NCBI Genbank nucleotide sequence database (Table S1). Hence, it was again determined that the Y03 isolate was an *A. baumannii*.

Genome functional component analysis

The protein coding genes of Y03 were predicted using the GeneMarkS software. The results showed that the protein coding genes of Y03 were 3 665 on the chromosome, 107 on the plasmid 1, 15 on the plasmid 2, 14 on the plasmid 3, and 7 on the plasmid 4, respectively (Table 2). The number of tRNA on the chromosome was 73, rRNA was 6, and ncRNA was 34. The number of CRISPRs on the chromosome was 3. Additionally, the interspersed repeats of Y03 on the chromosome included 9 SINEs (short interspersed repeats), 31 LINEs (long interspersed repeats), 72 L (long terminal repeats), 30 transposons, 9 unclassified interspersed repeats, and 4 satellites RNA (Table 2).

Genome subsystem analysis

Prophages of Y03 were predicted using the PHASTER tool. The results showed that there were 4 prophages on the chromosome, 1 prophage on the plasmid 1, and the plasmid 2, 3, and 4 had no prophage (Table 2). The number of GIs were 11 on the chromosome by comparison with the IslandViewer 4 database, and no GIs were predicted on the plasmid (Fig. 2F). The number of the virulence factors on the chromosome were 135 by BLAST analysis using the VFDB database, which were related with effector delivery system, immune modulation, adherence, stress survival, biofilm, exotoxin, and nutritional/metabolic factor (Table S2). There were 41 antibiotic resistance genes, 22 antibiotic target genes, and 3 antibiotic biosynthesis genes on the chromosome by BLAST analysis using the CARD database (Table S3).

Fig. 1 Isolation and identification of *A. baumannii* Y03. **A** *A. baumannii* was cultured on LB agar plates; **B** Observe the results under a 100-fold oil lens of microscope after gram staining; and **C** Electropherograms of *parC* in *A. baumannii* Y03. 2KM, 2 000 bp Marker; NC, blank control

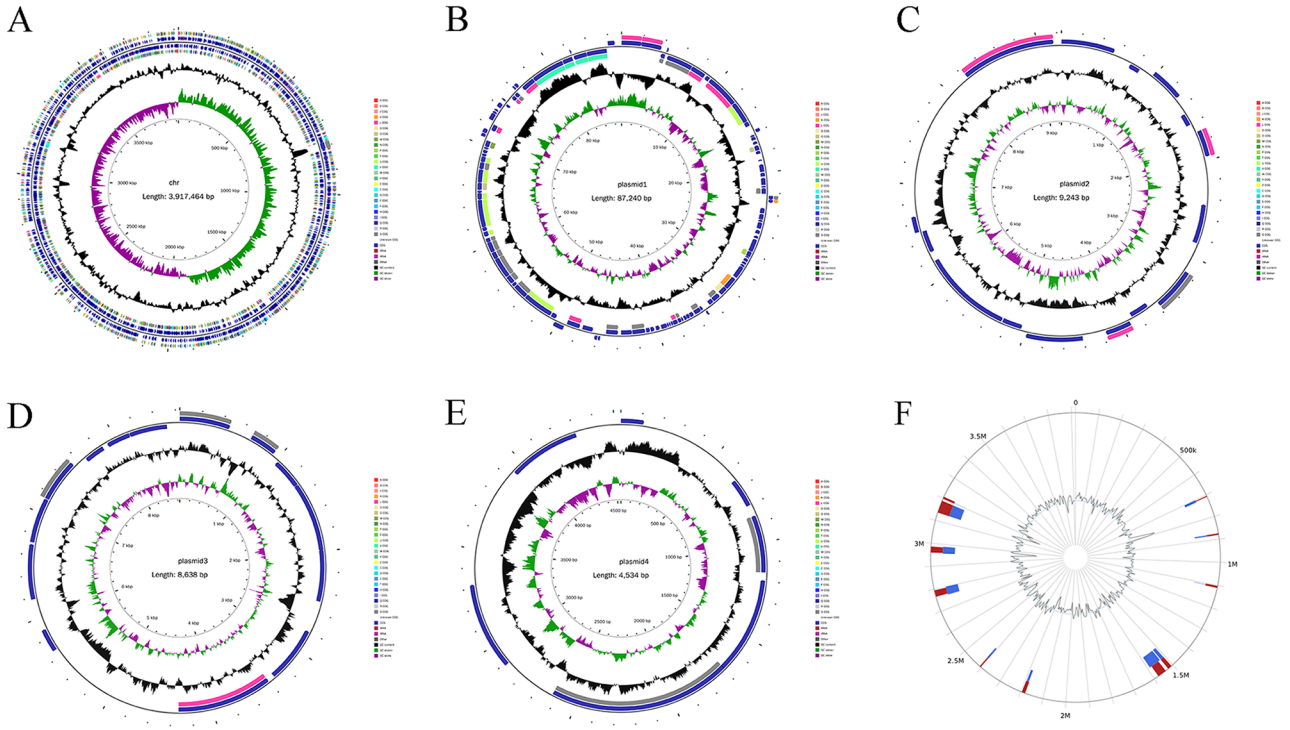

Fig. 2 Overview of the complete *A. baumannii* Y03 genome. **A** Chromosome; **B** Plasmid 1; **C** Plasmid 2; **D** Plasmid 3; and **E** Plasmid 4. From inner circle to outer circle, the first circle is the scale, the second circle is GC skew, the third circle is the GC content, the fourth and seventh circles are each ORF belonging to the COG classifications, and the fifth and sixth circles are the positions of ORF, tRNA, and rRNA on the genome. **F** Genomic islands (GIs) on the *A. baumannii* Y03 chromosome. Red is the predicted result by at least one method, blue is the predicted result by IslandPath-DIMOB, and yellow is the predicted result of SIGI-HMM

No antibiotic resistance genes were predicted on the plasmid. Additionally, a total of 85 carbohydrate-active enzymes on the Y03 genome were annotated using the CAZy database (Fig. 3 and Tables S4, 5), including 16 glycoside hydrolases (GHs), 20 glycosyl transferases (GTs), 2 polysaccharide lyases (PLs), 30 carbohydrate esterases (CEs), 11 auxiliary activities (AAs), and 4 carbohydrate-binding modules (CBMs) on the chromosome, and 2 GHs on the plasmid 1.

Genome functional annotation analysis

The protein coding genes were annotated using various databases. As shown in Table 3, the number of the protein coding genes annotated using the NR database was 3 657 on the chromosome, 106 on the plasmid 1, 13 on the plasmid 2, 14 on the plasmid 3, and 6 on the plasmid 4. By comparative analysis in the COG database, a total of 3 131 genes were annotated on the chromosome, 44 genes on the plasmid 1, 4 genes on the plasmid 2, 4 genes on the plasmid 3, and 2 genes on the plasmid 4 (Fig. 4; Table 3). As shown in Fig. 4A, 181 genes were related with cell wall/membrane/envelope biogenesis, 89 genes were related with signal transduction mechanisms, and 46 genes were related with defense mechanisms on the chromosome. 1 gene was related with cell wall/membrane/envelope biogenesis, 0 gene was related with signal transduction mechanisms, and 4 genes were related with defense mechanisms on the plasmid 1 (Fig. 4B).

The protein encoding genes on the Y03 genome were annotated and mapped into 8 KEGG categories by KO (KEGG ortholog) annotation and the KEGG pathway annotation. As shown in Fig. 5A, a total of 47 pathways on the chromosome were covered 8 KEGG categories, including brite hierarchies (3), cellular processes (4), environmental information processing (2), human diseases (10), genetic information processing (4), metabolism (12), not included in pathway or brite (4), and organismal systems (8). Similarly, 5 pathways on the plasmid 1 were covered 3 KEGG categories, including brite hierarchies (2), environmental information processing (2), and not included in pathway or brite (2); and 1 pathways on the plasmid 2 was not included in pathway or brite (Fig. 5B, C). The 4 most represented pathways on the chromosome related with survival were antimicrobial drug resistance (36), bacterial infectious diseases (21), environmental adaptation (8), and the host immune system (6) (Fig. 5A).

GO is a functional classification system fully describing the properties of genes and gene products [27]. In this

Table 2 General features obtained from *A. Baumannii* Y03 using whole-genome sequencing

Genomic parameter[a]	Value for parameter
Chromosome	
Accession number	CP163382
Sequence length/bp	3 917 464
GC content/%	38.92
ORF number	3 665
tRNA copy number	73
rRNA copy number	6
ncRNA copy number	34
Prophage number	4
Short interspersed repeats number	9
Long interspersed repeats number	31
Long terminal repeats number	72
Transposons number	30
Unclassified interspersed repeats number	9
Satellites RNA number	4
Simple-repeats number	0
Plasmid 1	
Accession number	CP163383
Sequence length/bp	87 240
GC content/%	34.01
ORF number	107
tRNA copy number	0
rRNA copy number	0
ncRNA copy number	0
Prophage number	1
Short interspersed repeats number	0
Long interspersed repeats number	0
Long terminal repeats number	0
Transposons number	0
Unclassified interspersed repeats number	0
Satellites RNA number	0
Simple-repeats number	0
Plasmid 2	
Accession number	CP163384
Sequence length/bp	9 243
GC content/%	34.70
ORF number	15
tRNA copy number	0
rRNA copy number	0
ncRNA copy number	0
Prophage number	0
Short interspersed repeats number	0
Long interspersed repeats number	0
Long terminal repeats number	0
Transposons number	0
Unclassified interspersed repeats number	0
Satellites RNA number	0
Simple-repeats number	0
Plasmid 3	
Accession number	CP163385
Sequence length/bp	8 638
GC content/%	31.86
ORF number	14

Table 2 (continued)

Genomic parameter[a]	Value for parameter
tRNA copy number	0
rRNA copy number	0
ncRNA copy number	0
Prophage number	0
Short interspersed repeats number	0
Long interspersed repeats number	0
Long terminal repeats number	0
Transposons number	0
Unclassified interspersed repeats number	0
Satellites RNA number	0
Simple-repeats number	0
Plasmid 4	
Accession number	CP163386
Sequence length/bp	534
GC content/%	37.21
ORF number	7
tRNA copy number	0
rRNA copy number	0
ncRNA copy number	0
Prophage number	0
Short interspersed repeats number	0
Long interspersed repeats number	0
Long terminal repeats number	0
Transposons number	0
Unclassified interspersed repeats number	0
Satellites RNA number	0
Simple-repeats number	0

ORF open reading frame, *ncRNA* non-coding RNA

[a]Based on NCBI Prokaryotic genomic annotation pipeline

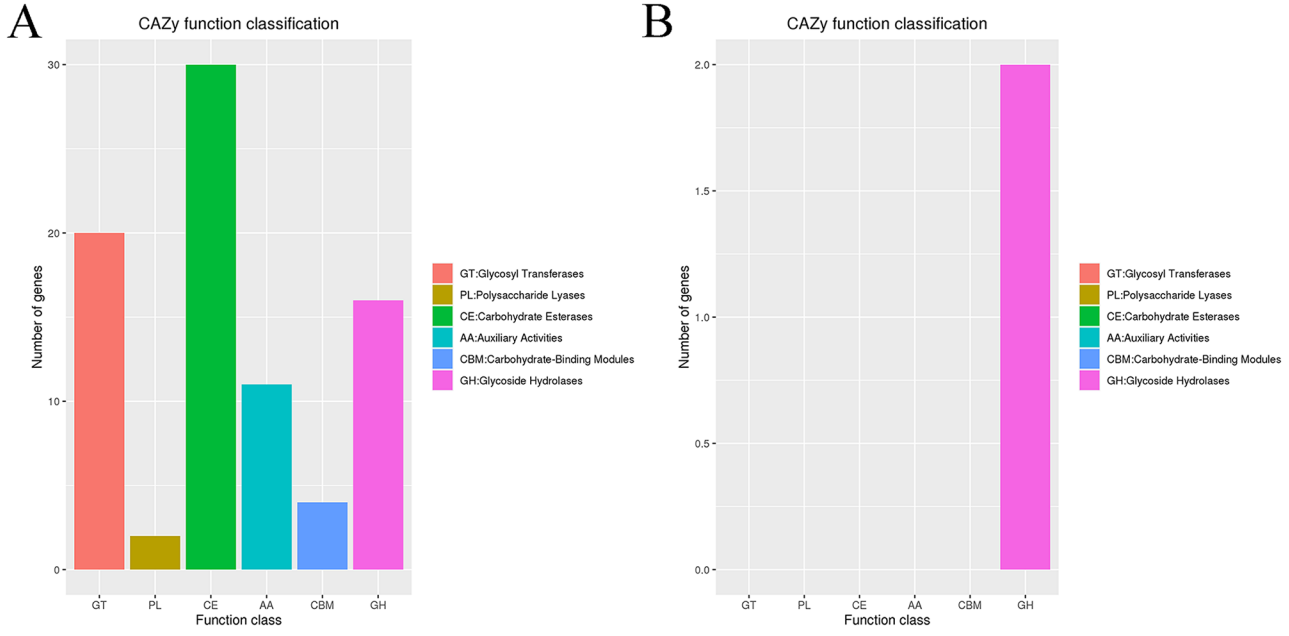

Fig. 3 The functional classifications annotated using CAZy on the *A. baumannii* Y03 genome. **A** Chromosome; and **B** Plasmid 1

Table 3 Overview on the functional annotation of the protein coding genes in *A. Baumannii* Y03

Sequence ID	Annotation in database	Genes number
Chromosome	NR	3 657
	COG	3 131
Plasmid 1	NR	106
	COG	44
Plasmid 2	NR	13
	COG	4
Plasmid 3	NR	14
	COG	4
Plasmid 4	NR	6
	COG	2

study, GO classification statistics revealed that a total of 73 GO terms on the chromosome were functionally characterized into 3 ontologies, including biological processes (38), cellular components (11), and molecular functions (24); 12 GO terms on the plasmid 1 included biological processes (4), cellular components (2), and molecular functions (5); 12 GO terms on the plasmid 2 included biological processes (4), cellular components (2), and molecular functions (6); 9 GO terms on the plasmid 3 included biological processes (5), cellular components (2), and molecular functions (2); and 1 GO terms on the plasmid 3 included biological processes (1) (Fig. 6). As shown in Fig. 6A, GO term related with stress response had 116 protein encoding genes, GO term related with cell wall organization or biogenesis had 41 protein encoding genes, and GO term related with cell adhesion had 7 protein encoding genes, etc.

A total of 647 transport proteins on the chromosome were obtained by comparative analysis of the TCDB database, including 109 channels or pores, 205 primary active transporters, 233 electrochemical potential-driven transporters, and 13 group translocators, 4 transmembrane electron carriers, 4 accessory factors involved in the transport, and 75 incompletely characterized transport systems (Fig. 7A). Moreover, there were 7 primary active transporters on the plasmid 1 (Fig. 7B).

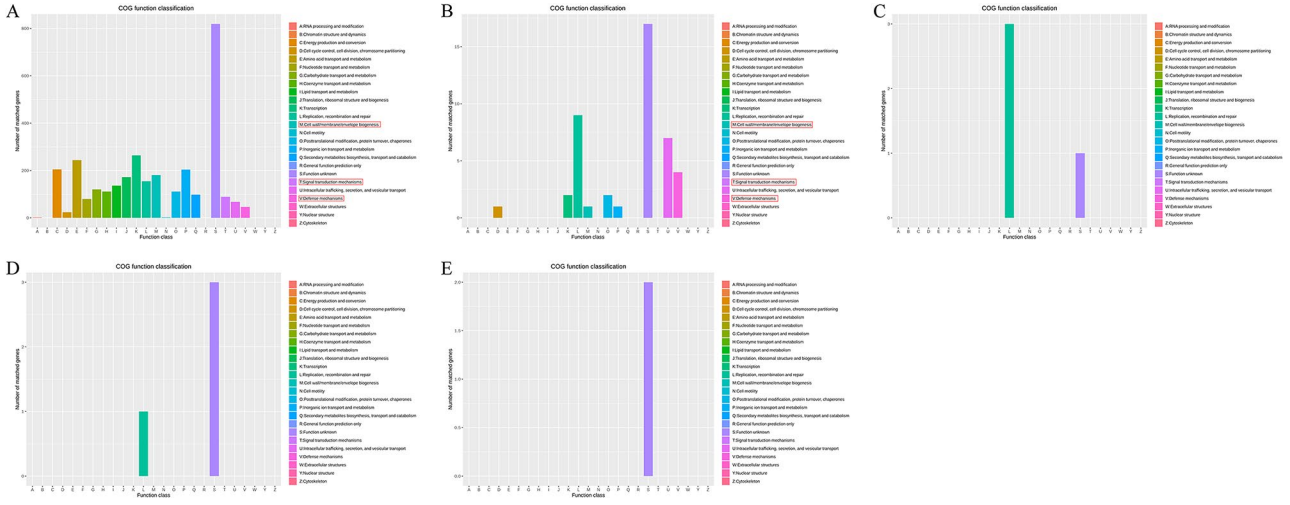

Fig. 4 The functional classifications annotated using COG on the *A. baumannii* Y03 genome. **A** Chromosome; **B** Plasmid 1; **C** Plasmid 2; **D** Plasmid 3; and **E** Plasmid 4

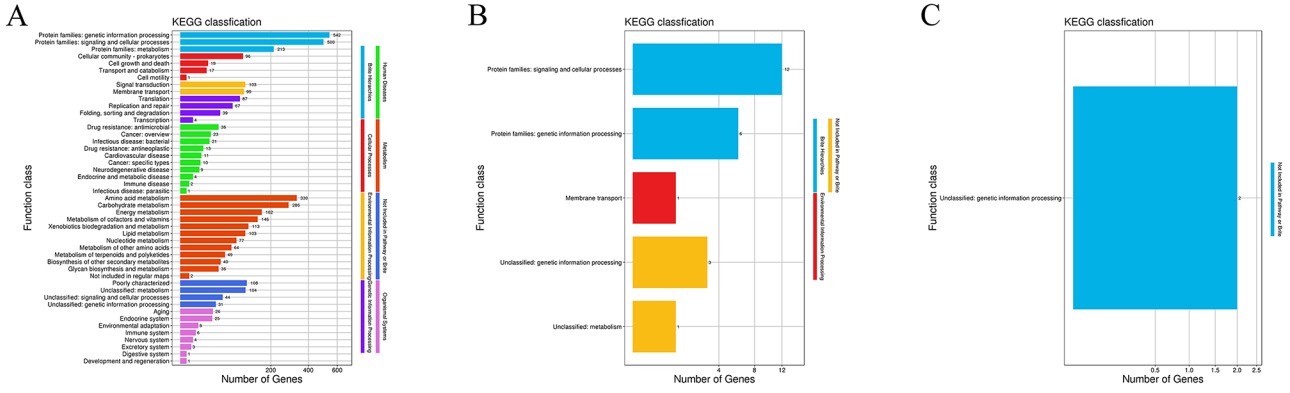

Fig. 5 The functional classifications annotated using KEGG on the *A. baumannii* Y03 genome. **A** Chromosome; **B** Plasmid 1; and **C** Plasmid 2

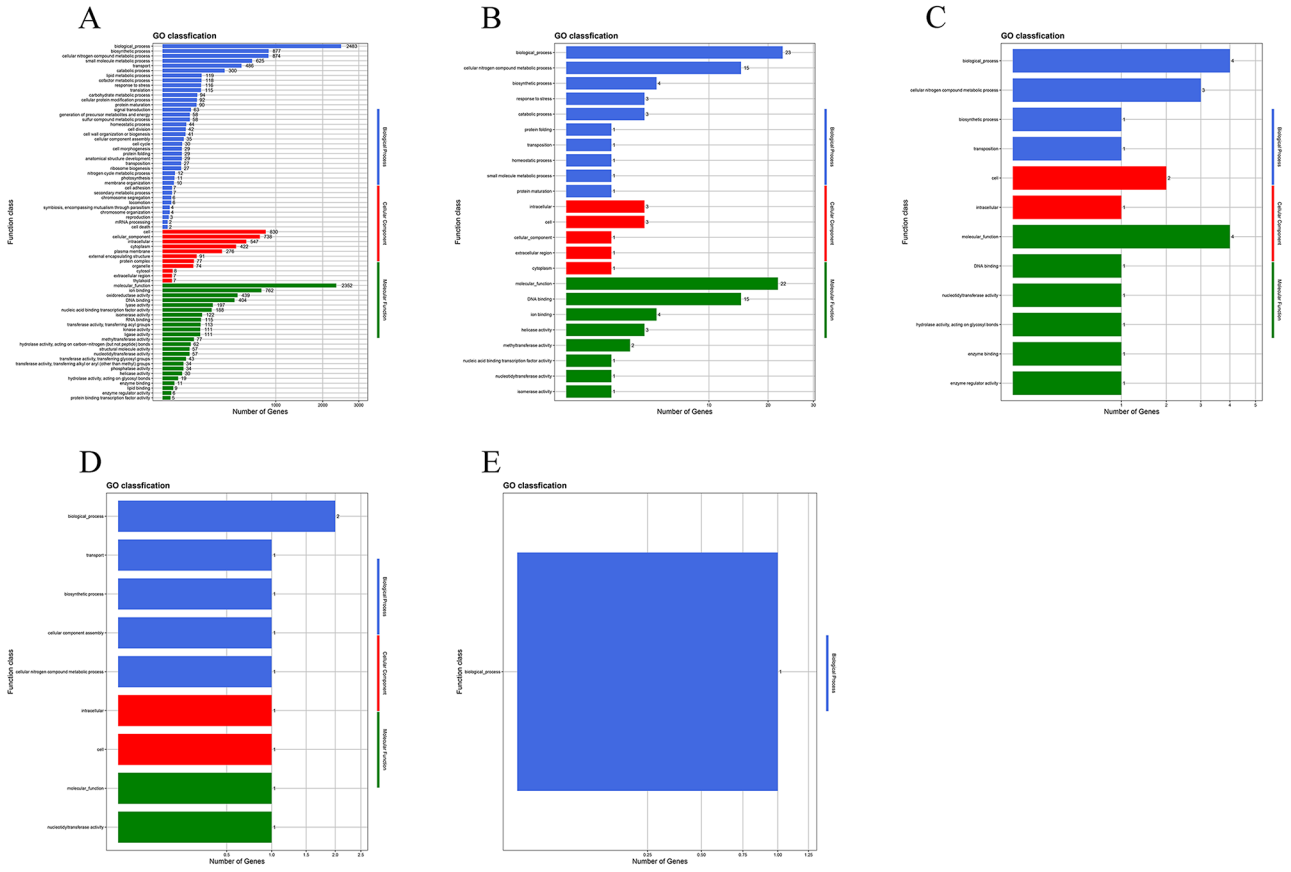

Fig. 6 The functional classifications annotated using GO on the *A. baumannii* Y03 genome. **A** Chromosome; **B** Plasmid 1; **C** Plasmid 2; **D** Plasmid 3; and **E** Plasmid 4

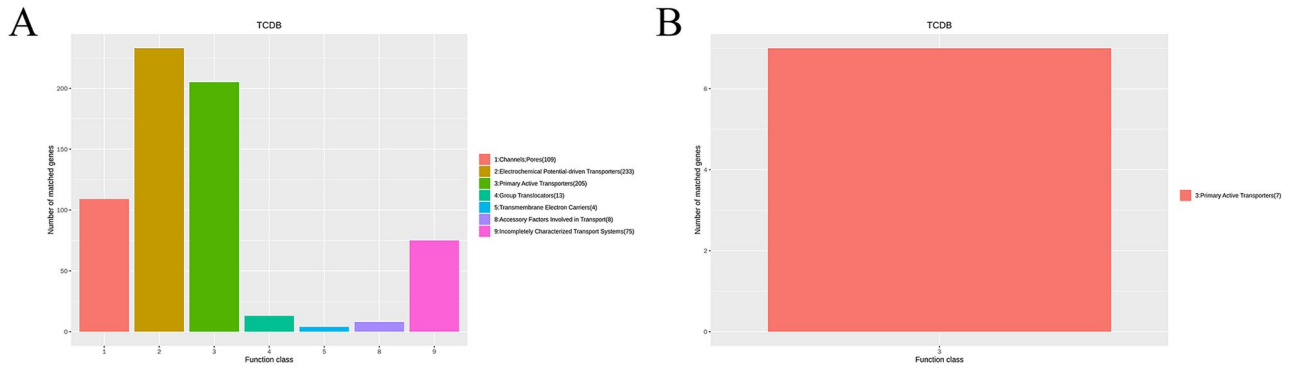

Fig. 7 The functional classifications annotated using TCDB on the *A. baumannii* Y03 genome. **A** Chromosome; and **B** Plasmid 1

The bacterial virulence and pathogenicity were annotated using the PHI database. The results revealed that 4 mutations were related with chemistry targets, 71 mutations were associated with enhanced virulence, 518 mutations leaded to reduced virulence, 44 mutations resulted in loss of pathogenicity, and 28 lethal mutations contributed to the death of Y03 on the chromosome (Fig. 8 and Table S6). The prediction results for the secreted proteins in Y03 indicated that a total of 264 secreted proteins were located on the chromosome, 12 secreted proteins on the plasmid 1, 1 secreted proteins on the plasmid 2, and no secreted proteins on the plasmid 3 and 4 (Table 4).

Antimicrobial susceptibility analysis

The antimicrobial susceptibility pattern of Y03 to the tested 34 antimicrobial agents was presented in Table 5 and Table S7. Y03 was resistant to penicillin G, ampicillin, oxacillin, cefalotin, cefazolin, cefoxitin, chloramphenicol, florfenicol, midecamycin, clindamycin, spectinomycin, and nitrofurantoin; intermediate

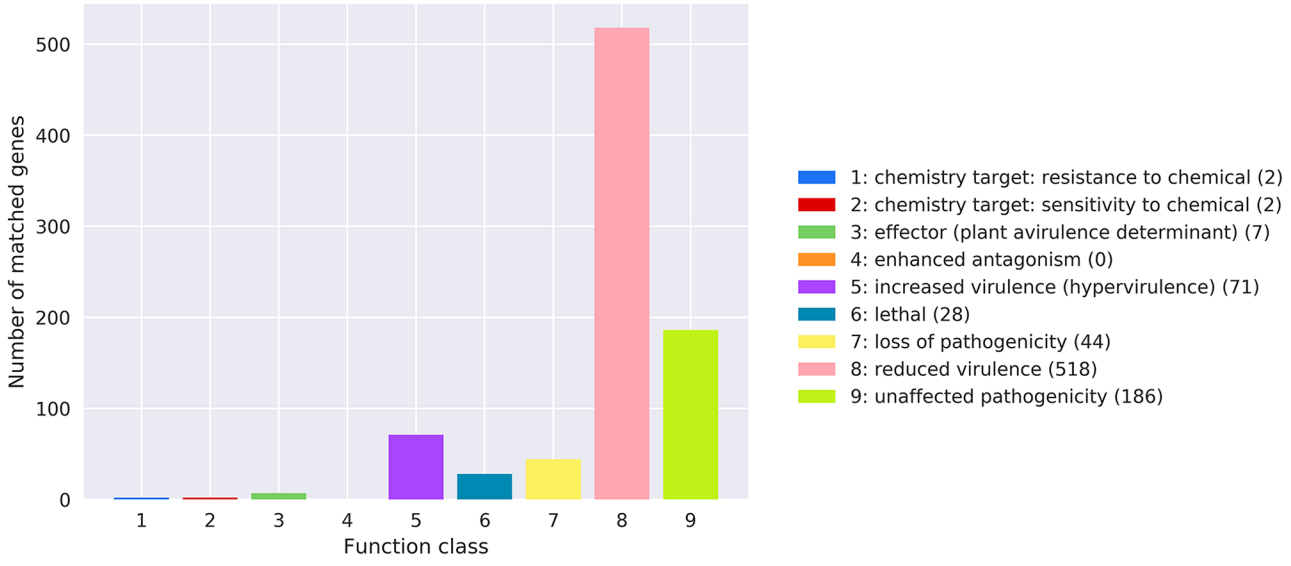

Fig. 8 The functional classifications annotated using PHI on the *A. baumannii* Y03 chromosome

Table 4 The prediction results of the secreted proteins in *A. Baumannii* Y03

Sequence ID	Secreted proteins number	Percentage[a]/%
Chromosome	264	7.20
Plasmid 1	12	11.21
Plasmid 2	1	6.67
Plasmid 3	0	0
Plasmid 4	0	0

[a]The secreted protein coding genes are as a percentage of the total protein coding genes

susceptible to aztreonam, cefotaxime, cefuroxime, clarithromycin, erythromycin, and tetracycline; and susceptible to piperacillin, cefoperazone, ceftazidime, cefepime, cotrimoxazole, ciprofloxacin, levofloxacin, ofloxacin, norfloxacin, minocycline, amikacin, tobramycin, streptomycin, gentamicin, kanamycin, and polymyxin B. These results demonstrated that Y03 was resistant to at least one agent from three or more antimicrobial classes and was considered to be a MDR *A. baumannii.*

Animal infection analysis

The virulence of Y03 was evaluated in the chick models. Chicks were intramuscularly infected with 2.0×10^{10}, 1.0×10^{10}, 1.0×10^{9}, 1.0×10^{8}, and 1.0×10^{7} CFU of Y03, and the chick mortality was observed for 7 days post-infection. On the first day, they began to show lethargy, anorexia and hypothermia in the test groups (group A, B, C, D and E) with Y03. Meanwhile, the mortality of chicks was observed in group A, B, and C, but not in group D, E, and the negative controls. As shown in Fig. 9, the mortality of group A was 100% (8/8), group B was 87.5% (7/8), and group C was 37.5% (3/8). Moreover, the histopathological sections of the Y03-infected group of chicks revealed that the myocardial fibers exhibited vacuolar degeneration, the interstitium was congested and edematous, and there was significant infiltration of heterophilic granulocytes in the heart (Fig. 10F). The hepatocytes were degenerated and necrotic, and numerous blue-staining granules in the hepatic sinusoids were suspected to be bacterial colonies in the liver (Fig. 10G). The necrosis and loss of lymphocytes in the white pulp were observed, along with infiltration of heterophilic granulocytes in the spleen (Fig. 10H). Congestion and inflammatory exudate in the bronchioles were observed, and the interstitium was widened and edematous in the lung (Fig. 10I). The tubular epithelial cells were degenerated and necrotic, and the interstitium was congested and hemorrhaged in the kidney (Fig. 10J).

Antimicrobial resistance genes and virulence genes

The antimicrobial resistance genes *abeM*, *adeB*, *adeH*, *adeK*, bla_{apmC}, bla_{OXA-90}, *catB9*, *macB*, *folP*, and *parE* were detected using PCR amplification method according to the results of antimicrobial susceptibility testing. As shown in Fig. 11A and Figure S1, Y03 contained *abeM*, *adeB*, *adeH*, *adeK*, bla_{apmC}, bla_{OXA-90}, *catB9*, *folP*, *macB*, and *parE*. The virulence genes included the *lpxC*, *pilO*, *fimT*, *ompA*, *basA*, *bauA*, *gspL*, *csu*, *pgaC*, *plc2*, *tssA*, *tviB*, *bap*, and *vgrG* on the Y03 chromosome (Fig. 11B and Figure S2).

Discussion

Multiple investigations have shown that the antimicrobial resistance mechanism and the pathogenic mechanism of *A. baumannii* as a nosocomial pathogen [4, 5, 28]. *A. baumannii* has been isolated from a variety of wild and domestic animals, in some cases, carbapenemase-producing isolates have been described [10, 29,

Table 5 Susceptibility of *A. Baumannii* Y03 to antimicrobial drugs

Antibiotics classes		Antimicrobial drugs	Inhibition zone diameter/mm	Susceptibility
β-lactams	Penicillins	Penicillin G	10.00 ± 1.00	R
		Ampicillin	11.67 ± 0.58	R
		Oxacillin	6.00 ± 0.00	R
		Piperacillin	21.67 ± 1.53	S
	Monobactams	Aztreonam	16.33 ± 1.15	I
	First-generation cephalosporins	Cefalotin	6.00 ± 0.00	R
		Cefazolin	6.00 ± 0.00	R
	Second-generation cephalosporins	Cefoxitin	12.33 ± 0.58	R
		Cefuroxime	16.00 ± 1.00	I
	Third-generation cephalosporins	Cefotaxime	17.00 ± 1.00	I
		Cefoperazone	18.00 ± 2.00	S
		Ceftazidime	18.33 ± 0.58	S
	Fourth-generation cephalosporins	Cefepime	21.00 ± 2.00	S
Sulfonamides		Cotrimoxazole	23.33 ± 2.89	S
Phenicols		Chloramphenicol	8.00 ± 1.73	R
		Florfenicol	15.00 ± 0.00	R
Macrolides		Midecamycin	6.00 ± 0.00	R
		Clarithromycin	17.00 ± 2.00	I
		Erythromycin	18.67 ± 0.58	I
Lincosamides		Clindamycin	6.00 ± 0.00	R
Fluoroquinolones		Ciprofloxacin	28.00 ± 1.00	S
		Levofloxacin	26.33 ± 2.08	S
		Ofloxacin	27.00 ± 2.00	S
		Norfloxacin	21.00 ± 1.00	S
Tetracyclines		Tetracycline	14.00 ± 2.00	I
		Minocycline	22.33 ± 2.51	S
Aminoglycosides		Amikacin	21.33 ± 2.89	S
		Tobramycin	23.67 ± 0.58	S
		Streptomycin	20.00 ± 0.00	S
		Gentamicin	20.33 ± 0.58	S
		Kanamycin	21.00 ± 1.73	S
		Spectinomycin	12.67 ± 1.15	R
Nitrofurans		Nitrofurantoin	6.00 ± 0.00	R
Polymyxins		Polymyxin B	12.67 ± 1.53	S

S susceptible, *I* intermediate susceptible, R resistant

30]. However, a study on the antimicrobial susceptibility profile and the pathogenicity of *A. baumannii* of chicken origin is rare, especially studies focusing on its genome. Therefore, understanding the genomic characteristics and the protein coding gene functions of *A. baumannii* could provide important insights into the adaptation and evolution of this bacterium of chicken origin.

Horizontal gene transfer refers to the transfer of the genetic material from one organism to another, significantly contributing to genome rearrangements and evolution [27, 31, 32]. The common horizontal gene transfer elements include transposons, prophages, plasmids, and GIs, which assist in the spread of antimicrobial resistance genes and virulence factors between bacteria, further increasing their antimicrobial resistance and pathogenicity [33–35]. There were 30 transposons, 5 prophages, 4 plasmids, and 11 GIs annotated on the genome of MDR *A. baumannii* Y03 using whole-genome sequencing, indicating that Y03 possessed the potential of these mobile genetic elements transferred to other bacteria and might be a significant threat to public health.

Whole-genome sequencing revealed that 3 CRISPRs were identified on the chromosome of MDR *A. baumannii* Y03, indicating that it could acquire some kind of adaptive immunity by integrating exogenous DNA into the chromosome via the spacer regions of CRISPRs [36, 37]. In addition, there are 3 131 protein encoding genes on the chromosome and 52 protein encoding genes on the plasmid, both of which have been annotated and functionally classified using the COG database. Among them, 842 protein encoding genes encode hypothetical proteins with unknown functions, which is a major hindrance in inferring their involvement in metabolism, proliferation, development, virulence, and antimicrobial resistance. Hence, further studies are needed to elucidate the precise function of these proteins.

Antimicrobial resistance is one of the most important characters of the *A. baumannii* isolates [6]. We found that MDR *A. baumannii* Y03 was resistant to several β-lactams, phenicols, macrolides, lincosamides, aminoglycosides, and nitrofurans. There were 41 antimicrobial resistance genes identified on the Y03 chromosome using whole-genome sequencing, including (i) β-lactamses: bla_{AmpC} and bla_{OXA-90}; (ii) the ATP-binding cassette (ABC) transporters: *macB*; (iii) the resistance nodulation cell division (RND) family: *adeAB*, *adeFGH* and *adeIJK*; (iv) the small multidrug resistance (SMR) family efflux pump: *abeS*; (v) the multidrug and toxic compound extrusion (MATE) family: *abeM*; (vi) chloramphenicol acetyltransferase: *catB9*; (vii) the resistance genes to fluoroquinolones: *gyrA*, *parE*, and *parC*; and others. Various studies have demonstrated that many multidrug efflux pump systems are correlated with resistance to a range of antibiotics in other *A. baumannii* strains, including aminoglycosides, β-lactams, tetracyclines, and fluoroquinolones [38–40]. However, Y03 was susceptible to most of aminoglycosides, tetracyclines, fluoroquinolones, sulfonamides, polymyxins, third-generation and fourth-generation cephalosporins. This suggests that the resistance

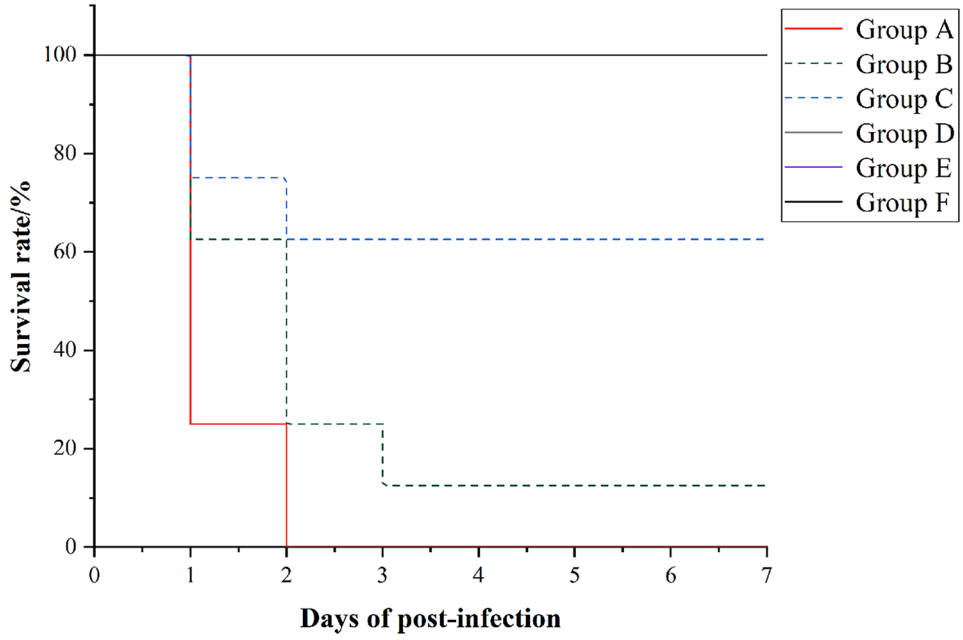

Fig. 9 The survival rates of chicks infected by different concentrrations of *A. baumannii* Y03 were detected using animal infection experiments. Seven-day-old chicks in the test groups were intramuscularly injected with 2.0×10^{10}, 1.0×10^{10}, 1.0×10^{9}, 1.0×10^{8}, and 1.0×10^{7} CFU of MDR *A. baumannii* Y03. Seven-day-old chicks in the control groups were intramuscularly injected with PBS. Survival was monitored until 7 days post-infection

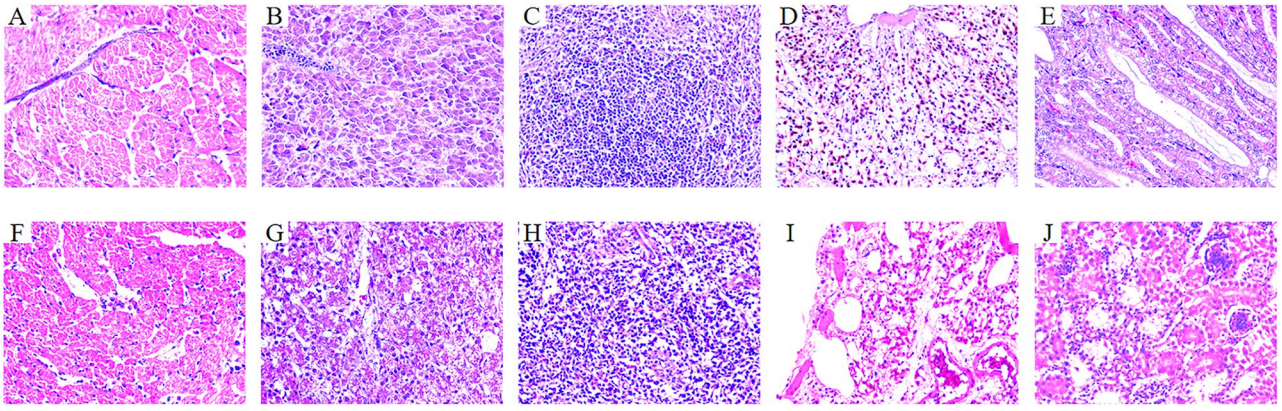

Fig. 10 Histological observation of chick lesions (400×). **A**–**E** Heart, liver, spleen, lung, and kidney of group F, which was the control group of chicks intramuscularly injected with PBS; and **F**–**J** Heart, liver, spleen, lung, and kidney of group E, which was the Y03-infected group of chicks intramuscularly injected with 1.0×10^{7} CFU of MDR *A. baumannii* Y03

mechanisms of Y03 are different from those of other *A. baumannii* strains, and some antimicrobial resistance genes of Y03 might be less active or expressed at lower levels compared to their homologs in other *A. baumannii* strains, and they only constitute the intrinsic resistome of Y03. Furthermore, there were 22 antibiotic target genes on the Y03 chromosome encoding the B subunit of DNA topoisomerase IV, the β subunit of RNA polymerase, the A subunit of DNA gyrase, and others. This implies that certain antibiotics could be used to kill MDR *A. baumannii* Y03, including fluoroquinolone, cycloserine, and rifamycin.

A total of 135 virulence genes belonging to 17 classes of virulence factor associating with the pathogenic potential of Y03 were identified using whole-genome sequencing, including adhesins, invasion, biofilms, acinetobactin, secretion proteins, toxins, cell surface appendages, immune evasion, serum resistance, stress adaptation, and others. And we certificated that Y03 was virulent to chickens in the in vivo infection experiments; however, it did not exhibit comparable hypervirulence, which is defined as $\leq1.0\times10^{8}$ CFU of bacteria causing the death of experimental animals. Although *A. baumannii* ATCC 19606 only contained 69 virulence genes associated with adherence, biofilm formation, phospholipase,

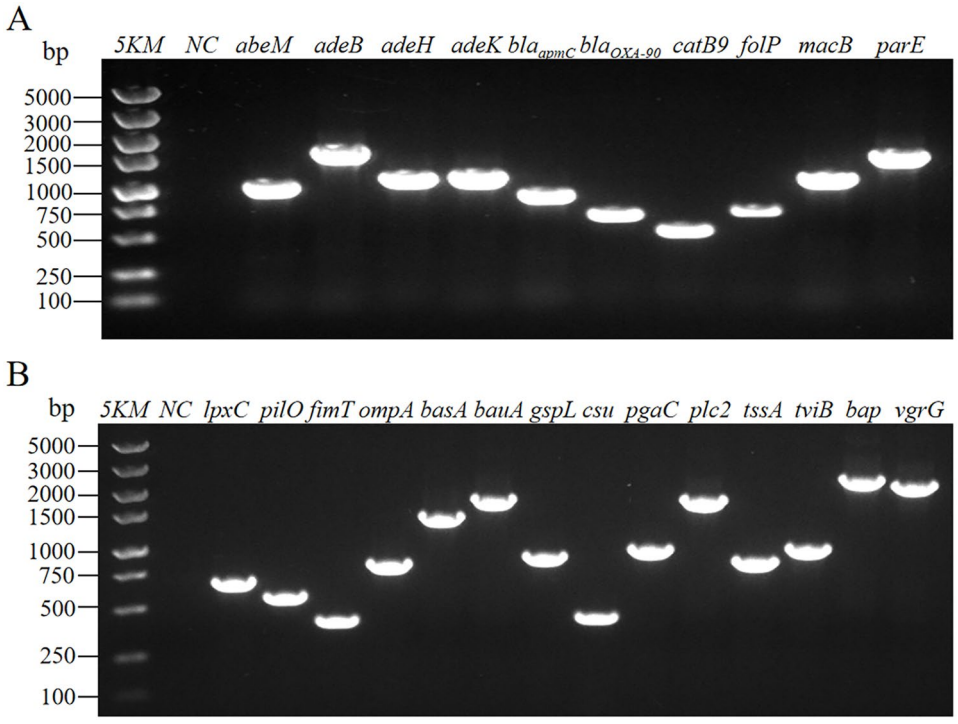

Fig. 11 Electropherograms of antimicrobial resistance genes and virulence genes in MDR *A. baumannii* Y03. **A** Electropherograms of antimicrobial resistance genes in *A. baumannii* Y03. 5KM, 5 000 bp Marker; NC, blank control. **B** Electropherograms of virulence genes in *A. baumannii* Y03. 5KM, 5 000 bp Marker; NC, blank control

iron uptake, and immune evasion, 1.9×10^6 CFU of ATCC 19606 resulted in a mortality of 100%, illustrating that it exhibited hypervirulence in a mouse infection model [38, 41, 42]. Therefore, this suggests that the pathogenicity and the pathogenic mechanisms of MDR *A. baumannii* Y03 in a chickens infection model is complex and differs from that of *A. baumannii* ATCC 19606 in a mouse infection model. Besides, there were 518 mutations leading to reduced virulence, 44 mutations resulting in loss of pathogenicity, and 28 lethal mutations contributing to the death of Y03 using the PHI database annotation. This implies that certain mutants could reduce virulence or lose pathogenicity, and even cause the death of MDR *A. baumannii* Y03.

Our study has some limitations. Our results from the study demonstrated that MDR *A. baumannii* Y03 contained 41 antimicrobial resistance genes on its chromosome, but Y03 was only resistant to 6 classes of antimicrobial agents, including several β-lactams, phenicols, macrolides, lincosamides, aminoglycosides, and nitrofurans. Consequently, the discrepancies between the phenotype and genotype of antimicrobial resistance remains to be fully explored. Despite the high carriage rate of virulence factors, Y03 did not exhibit comparable hypervirulence in the in vivo chickens infection experiments. Therefore, it is necessary to confirm whether inherent or chromosomal mechanisms underlie the observed virulence heterogeneity in the MDR *A. baumannii* Y03 in future experiments.

In reference to future perspectives, extensive epidemiological researches are essential to comprehensively investigate the distribution of *A. baumannii* isolates in food-producing animals, their antimicrobial resistome, their virulence factor and the pathogenicity, and their association with *A. baumannii* of human origin.

Conclusion

This study reported that MDR *A. baumannii* Y03 isolated from chickens contained 1 circular chromosome harboring 41 antimicrobial resistance genes and 135 virulence genes, as well as 4 circular plasmids. Y03 poses a critical threat to global health due to its virulence and resistance to 6 classes of antibiotics, including several β-lactams, phenicols, macrolides, lincosamides, aminoglycosides, and nitrofurans. Interestingly, whole-genome sequencing analysis revealed that 22 antibiotic targets and 28 lethal mutations on the Y03 chromosome could serve as potential targets to prevent, control, and treat infections caused by MDR *A. baumannii* Y03.

Abbreviations

A. baumannii	Acinetobacter baumannii
MDR	Multidrug-resistant
XDR	Extensively drug-resistant
PDR	Pan-drug-resistant

ncRNA	Non-coding RNA
rRNA	Ribosome RNA
sRNA	Small RNA
rRNA	Ribosome RNA
tRNA	Transfer RNA
snRNA	Small nuclear RNA
snoRNA	Small nucleolar RNA
miRNA	microRNA
NR	Non-Redundant Protein Database
GO	Gene Ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
COG	Clusters of Orthologous Groups
TCDB	Transporter Classification Database
CAZy	Carbohydrate-Active Enzymes Database
PHI	Pathogen Host Interactions Database

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12866-024-03694-7.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Supplementary Material 6

Supplementary Material 7

Supplementary Material 8

Supplementary Material 9

Supplementary Material 10

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Author contributions
LY: investigation, methodology, experiments, writing original draft, writing review and editing, data curation and funding acquisition. SZ, WN and YZ: methodology and experiments. LZ, CX and PK: formal analysis, methodology. XZ: conceptualization, supervision and funding acquisition. All authors read and approved the final manuscript.

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Data availability
The complete genome sequences of MDR A. baumannii Y03 including 1 chromosome sequence and 4 plasmids sequences using whole-genome sequencing have been deposited into the NCBI Genbank nucleotide sequence database under accession numbers CP163382, CP163383, CP163384, CP163385, and CP163386. The raw sequencing data of MDR A. baumannii Y03 are available in the NCBI Sequence Read Archive (SRA) database with the SRA accession number SRR30247697.

Declarations

Ethics approval and consent to participate
This study was carried out in compliance with the ARRIVE guidelines. Animal experiments were conducted under animal protocols approved by the Institutional Animal Care and Use Committee (IACUC) of Linyi University (Approval No. LYU20240206). All animal work was carried out following accordance within the guidelines of the Laboratory Animal Research Center of Linyi University.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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