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RESEARCH ARTICLE

Acinetobacter of Pigs Reveals High Multiple Drug Resistance Through Genomics and Antimicrobial Resistance Monitoring

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ABSTRACT

Acinetobacter is an important opportunistic pathogen associated with severe infections in humans and animals worldwide. In veterinary medicine, the resistance patterns of *Acinetobacter* species remain unclear, with limited information available. This study examined the genomics characterization and antimicrobial resistance of *Acinetobacter* strains from swine industry of Shanxi province in China. The analysis of core genome phylogenetic and antibiotic genetic determinants from *Acinetobacter* has shown that the number of specific core genes varied from 105 to 293, with TG9 as an outlier. Functional gene annotation from COG, GO, and KEGG analyses revealed high consistency, particularly in genes related to amino acid transport, metabolism, transcription, and energy production. Meanwhile, these strains exhibited the endemic characteristics of *Acinetobacter spp*., as well as the close evolutionary relationships of antibiotic resistance genes. All isolated strains had a high multidrug resistance (50%), which highlights their pathogenic for oxacillin (79.2%), cefazolin (41.7%), cotrimoxazole (50%), and tetracycline (25%). Upon treatment with ampicillin, cefotaxime, and sulfonamides, the expression of *OXA51*, *AmpC*, *abeM*, *abeS*, *TEM*, and *sul2* mRNA in various specific *Acinetobacter* strains were elevated to different extents, particularly pronounced upregulation in *A. baumannii*. This study significantly advances our understanding of antibiotic resistance in foodborne *Acinetobacter*. It provides valuable theoretical insights for controlling the spread of *Acinetobacter* species and reducing the associated public health risks.

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INTRODUCTION

Acinetobacter spp. infections are a significant public health concern worldwide and are relatively common in animals, particularly in livestock and pets (Wareth et al., 2019). Similar to human healthcare, the misuse of antibiotics in veterinary medicine and livestock farming contributes to seriously antibiotic resistance in animal *Acinetobacter spp.*, increasing the risk of transmission to humans in close-contact behavior like homes and farms (Maboni et al., 2020). Therefore, understanding the epidemiology, mechanisms of resistance, developing new diagnostic tools and vaccines are important for effectively controlling and preventing infections of *Acinetobacter spp*. (Ranjbar and Alam, 2023). Currently, *Acinetobacter* includes 82 species and poses a threat to public health due to its increasing resistance to all antimicrobial drugs (Govender et al., 2021). Within these *Acinetobacter* species, *Acinetobacter baumannii* represents as the most harmful strain, for its multidrug resistance or even pan-drug resistance, making it an infamous pathogen of infections (Ibrahim et al., 2021). Although carbapenems have traditionally been considered as potent antimicrobials against infections caused by multidrug-resistant (MDR) *Acinetobacter spp.*, their efficacy of therapy is increasingly compromised by antibiotic misuse and the evolution of resistance in *Acinetobacter* (Nguyen and Joshi, 2021).

Acinetobacter is less susceptible to multiple classes of antibiotics due to mechanisms including out membrane impermeability, efflux pump activity, and the chromosomal encoding of two β-lactamases. This resistance extends to various antibiotics including β-lactams, macrolides, trimethoprim, and fosfomycin, highlighting the complexity of managing *Acinetobacter* infections (Lee et al., 2017). β-lactamases as the

extended-spectrum β-lactamases to cefotaxime-resistant enzymes, are marked by resistance genes like *TEM* and *CTX-M*. (Shapiro, 2017). Moreover, the role of multidrug and toxic transporters *abeM* and *abeS*, in conferring resistance to *Acinetobacter* against quinolones remains under debate. A critical element defined as ATPase *MacB* plays a synergistic role in resistance to macrolide antibiotics (Leus et al., 2018). Tetracycline antibiotics, which target ribosomal subunit to inhibit the initiation of translation, encounter resistance mechanisms, particularly through the *tetA* and *tetB*, which appear to facilitate the tigecycline-related efflux entering cytoplasm (Cheng et al., 2022; Wen et al., 2020). Moreover, *sul1* and *sul2* also have been proven to be indispensable factors in regulating resistance to sulfonamides in *Acinetobacter spp*. (Abdi et al., 2020). Effective monitoring antibiotic resistance of *Acinetobacter* is crucial to avoiding a global health crisis.

This study aims to elucidate the antibiotic resistance patterns of *Acinetobacter* isolated from pigs in China, focusing on core genes and resistance gene distribution. Furthermore, we explored multidrug resistance with susceptibility assay and analyzed resistance gene expression in response to antibiotics. The insights are expected to enhance our understanding of antibiotic resistance in foodborne *Acinetobacter*, expecting to contribute to valuable information for further establishment of clinical breakpoints for susceptibility testing in animal-associated *Acinetobacter* isolates.

MATERIALS AND METHODS

Resuscitation of strains, genome sequencing and data assembly: The 24 strains, which were isolated by lungs from swine industries in Shanxi province of China in 2022, were stored with glycerol at -80℃ at the Shanxi Agricultural University and reactivated onto a nutrient agar medium at 37℃ for 18h. The total DNA was extracted and the draft genomes of 24 *Acinetobacter* strains were performed to analyze sequence using the Illumina HiSeq × 10 sequencing platform as described previously (Zhang et al., 2019). The sequences of the isolates (Accession number: PRJNA1069300) were analyzed by comparing them with NCBI.

Core-genome and annotation analysis: The PanGP-v1.0.1 software was used to fit and visualize core gene (Zhao et al., 2014). Ortholog software was employed to make a diagram of the specific genome of these strains. For protein functional classification, Blastp-v2.9.0+ with the E-value threshold of 1e-20 searching against the COG database was employed. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) were performed to characterize gene properties and provide comprehensive functional annotation of the genome or transcriptome of newly sequenced species.

Identification and analysis of antibiotic resistance genes: The antibiotic resistance genes of 24 isolated strains were predicted by aligning assembled genome sequence against the Comprehensive Antibiotic Research Database (CARD). All antimicrobial resistance genes were classified and obtained comprehensive list according to gene categories. Statistical analysis results are presented by heatmap by GraphPad Prism 9.0.1 software.

Antimicrobial susceptibility test: The antimicrobial susceptibility profile of isolates was verified by the Kirby-Bauer disc diffusion method after incubation for 24h and the results were interpreted according to CLSI guidelines. The types and concentrations of 13 drug-resistant drugs are shown in Fig 2A. In this study, the MDR is defined as resistance to at least one antimicrobial agent in three or more antibiotic classes.

Genomic DNA extraction and Quantitative real-time PCR: All strains were harvested after resuscitation and total bacterial DNA was extracted by boiling method (Li et al., 2023). The PCR products were verified by DNA sequencing with primers in Table 1. To clarify the causes of resistance to ampicillin, cefazolin, and sulfonamides isolates of *Acinetobacter spp.*, the expression levels of the relevant resistance genes were examined following 5 represented strains in the presence of different concentrations of the drugs by Q-PCR. mRNA analysis was performed following the previous protocol (Wu et al., 2021). Primer sequences are shown in Table 2.

Table 1: PCR primers.

Gene Primer ID	Primer sequence (5'-3')
*sul2*_F	TCGTCAACATAACCTCGGACAG
*sul2*_R	TTTCAGCGCCGCCAATAC
*tetA*_F	GCTACATCCTGCTTGCCTTC
*tetA*_R	CATAGATCGCCGTGAAGAGG
*CTX-M-1*_F	ATGGTTAAAAAATCACTGCGYCAGTTC
*CTX-M-1*_R	TCACAAACCGTYGGTGACGATTTTAGCCGC
*MacB*_F	TACTAAAACGCAAAACCGACCA
*MacB*_R	CATCACTTCAACGCCGCTA

Table 2: Q-PCR primers.

Gene Primer ID	Primer sequence (5'-3')
*sul2*_qPCR_F	ATGAAGTCAGCTCCACCTGC
*sul2*_qPCR_R	TTCGCGCAAATCCTTTCTGC
*tetA*_qPCR_F	GCTCGTGGGCTGATGG
*tetA*_qPCR_R	CTTTGTGCGACTCCGGC
*MacB*_F	AATGAATGGCGGCGATGTA
*MacB*_R	GTGAATCGAGTGCCCCTGTT
*OXA51*_qPCR_F	AGGAAGTGAAGCGTGTTGGT
*OXA51*_qPCR_R	TGGATTGGAACTCATCTTGGAC
*AmpC*_qPCR_F	GGCTCAACCAACGGTTTCGG
*AmpC*_qPCR_R	ACGCTGCCTTAATGCGCTCT
*abeM*_qPCR_F	AGCAATTTCAGTCACTTCGGTA
*abeM*_qPCR_R	CTTTTCACCATAATACGTCCC
*adeJ*_qPCR_F	GCTTCACAATATGGCTTACGTT
*adeJ*_qPCR_R	CATCCCGAACAGTGATAGCG
*abeS*_qPCR_F	TGTGGGTTATGCAGTTGCTTTT
*abeS*_qPCR_R	GGCATAGGCAATCCCGATT
*TEM*_qPCR_F	CTCGGTCGCCGCATACACTA
*TEM*_qPCR_R	AGCGGTTAGCTCCTTCGGTC
*16S*_F	AGCTAACGCGATAAGTAGACG
*16S*_R	TGTCAAGGCCAGGTAAGGTTC

Statistical analysis: All statistical analyses were performed using GraphPad Prism 9 (GraphPad Inc., CA, USA). The data were presented as means ± SE. Comparisons between 2 groups were performed by unpaired Student's test. Statistical significance was defined as $P < 0.05$.

RESULTS

Genome analysis and antibiotic resistance gene expression in *Acinetobacter*: A total of 24 isolated *Acinetobacter* strains were identified, including *Acinetobacter baumannii* (WX9), *Acinetobacter junii*

(GP3, GP6, GP8, GP10, and WX4), *Acinetobacter lwoffii* (YQ5, TG9, PY4, GP1, GP9, and YZ5), *Acinetobacter ursingii* (YZ6, YZ7, and YZ22), and *Acinetobacter towneri* (QW5, WS5, YP12, GP4, YZ23, YZ24, YZ3, LY2, and LY8). Most strains lacked multi-copy core genes, except WX9, TG9, YZ22, WS5, YZ23, YZ24, and YZ3. The number of specific core genes per *Acinetobacter* strain varied from 105 to 293, with TG9 as an outlier (Fig. 1A). The addition of new strains indicated a notable increase in multi-copy core genes, underscoring the high intraspecific diversity within *Acinetobacter*, which may contribute to their widespread environmental presence. Functional gene annotation from COG, GO, and KEGG analyses revealed high consistency, particularly in genes related to amino acid transport, metabolism, transcription, and energy production (Fig. 1B). This underscores that a larger dataset of bacterial strains offers a more complete understanding of genomic features and genetic diversity. To assess antibiotic resistance genes in *Acinetobacter* isolates, we employed the CARD, specifically focusing on its Antibiotic Resistance Ontology (ARO). This ontology encompasses terms related with ARGs, resistance mechanisms, antibiotics, and their targets. Furthermore, we analyzed the genomes of 24 *Acinetobacter* strains using Antibiotic Resistance Ontology. The results found the quinolone resistance gene *gyr* in all isolates. β-lactam resistance genes from the *OXA* group, such as *OXA23*, *OXA24*, *OXA51*, and *OXA58*, were identified in 37.5% of the isolates (9/24), accounting for approximately 60% of the total antibiotic resistance genes detected across all samples. Sulfonamide resistance genes *sul1*, *sul2*, and *sul3* were present in 41.6% of the strains (10/24). Tetracycline resistance genes, including *tetA*, *tetB*, and *tetD*, were observed in 62.5% of isolates. The *mex* gene was ubiquitous across all isolates, as were the efflux pump genes *abe* and *ade*. The *Mac* gene was detected in 91.6% of isolates (22/24). Significantly, the WX9 strain harbored all the resistance genes listed (Fig. 1C). These findings highlight the extensive and varied antibiotic resistance gene profiles in *Acinetobacter* isolates, indicating diverse drug resistance capabilities.

To further elucidate the basis of resistance, we analyzed the expression of antibiotic resistance and efflux pump genes via PCR assays. Our findings indicated that 41.6% of isolates harbored the $bla_{CTX\text{-}M\text{-}1}$ gene, which confers β-lactam resistance. The *sul2* gene was detected at a higher rate of 54.1%. Notably, the *tetA* gene was present in all *Acinetobacter* strains, reflecting a 100% detection rate. Additionally, the *MacB* gene was detected in 41.6% of the isolates, suggesting a moderate level of expression (Figure. 1D). These patterns indicate a correlation between gene expression and the observed antibiotic resistance profiles.

Antimicrobial resistance rates and multidrug-resistant phenotypes in *Acinetobacter* isolates: We assessed the antimicrobial resistance of *Acinetobacter* isolates to various drugs using comprehensive antibiotic resistance assays. This study revealed that the WX9 strain of *A. baumannii* showed resistance to 76.9% of the antibiotics tested (10/13), while the *A. lwoffii* YZ5 strain exhibited resistance to 53.8% (7/13) of antibiotics. Notably, intermediate resistance to certain antibiotics was recorded in *A. junii* WX4 (50%), *A. ursingii* YZ6 (53.8%), and *A. towneri* YZ23 (53.8%) (Fig. 2A). The MIC plate assays visually confirmed these resistance rates for selected isolates (Data not shown). An aggregate analysis of the 24 isolates showed varying resistance rates to the antibiotics tested, including ampicillin (12.5%), piperacillin (25%), oxacillin (79.2%), cefazolin (41.7%), ceftazidime (8.3%), cotrimoxazole (50%), tetracycline (25%), gentamicin (4.2%), erythromycin (8.2%), clindamycin (12.5%), polymyxin B (0%), ciprofloxacin (4.2%), and vancomycin (16.7%) (Fig. 2B). Further analysis indicated that 11 isolates (45.8%) were multidrug-resistant. Moreover, 9 strains showed resistance to at least one antibiotic class, and 4 strains were susceptible to all tested drugs (Fig. 2C). Overall, *A. baumannii* demonstrated the broadest spectrum of drug resistance compared to the other strains.

Resistance gene expression in response to different antibiotics in *Acinetobacter* isolates: In standard culture conditions, WX9 entered the logarithmic growth phase at 4h and reached the stationary phase by 8h. However, when cultured with 25μg/mL sulfamethoxazole, the logarithmic phase was delayed until 8h, with the stationary phase extending to 16h. YZ23 exhibited a similar growth pattern under these conditions (Data not shown). These insights suggest that resistant strains express resistance genes more robustly when cultured for 16h. Building on prior research, we investigated the influence of various antibiotic concentrations on the expression of resistance genes in isolates of *Acinetobacter spp.*, including *A. baumannii* (WX9), *A. lwoffii* (YZ5), *A. junii* (WX4), *A. ursingii* (YZ6), and *A. towneri* (YZ23). The WX9 strain, treated with 50μg/mL ampicillin, showed a significant increase in *OXA51* and *adeJ* genes expression compared to the untreated control. Additionally, the expression of *AmpC*, *MacB*, and *abeM* genes was consistently higher with increasing ampicillin concentrations. In contrast, ampicillin treatment led to the suppression of the *abeS* gene in WX9, which was generally more susceptible to antibiotics (Figure. 3A). With cefotaxime exposure, the expression levels of *OXA51*, *AmpC*, *abeM*, and *abeS* in WX9 were notably higher than the control, while *adeJ* expression remained unchanged (Figure. 3B). Sulfamethoxazole treatment caused an elevation in *sul2*, *MacB*, and *abeS* levels but did not affect *adeJ* gene expression (Figure. 3C). In *A. lwoffii* YZ5, ampicillin and cefotaxime significantly reduced *TEM* gene expression, while the *sul2* gene was upregulated by 25 and 50μg/mL ampicillin treatments (Figures. 3D and E). A 50μg/mL sulfamethoxazole treatment led to a considerable increase in both *TEM* and *sul2* gene expression (Figure. 3F). For *A. junii* WX4, 50μg/mL of ampicillin, cefotaxime, and sulfamethoxazole induced higher expression of *TEM* and *sul2*, except the 50μg/mL sulfamethoxazole group (Figures. 3G, H, and I). Conversely, in *A. ursingii* YZ6, ampicillin did not enhance *TEM* or *sul2* gene expression (Figure. 3J). Cefotaxime at 25μg/mL significantly increased *TEM* expression, and a 10μg/mL concentration raised *sul2* levels (Figure. 3K). Sulfamethoxazole preferentially upregulated *sul2* over *TEM* expression in YZ6 (Figure. 3L). Lastly, in *A. towneri* YZ23, ampicillin treatment

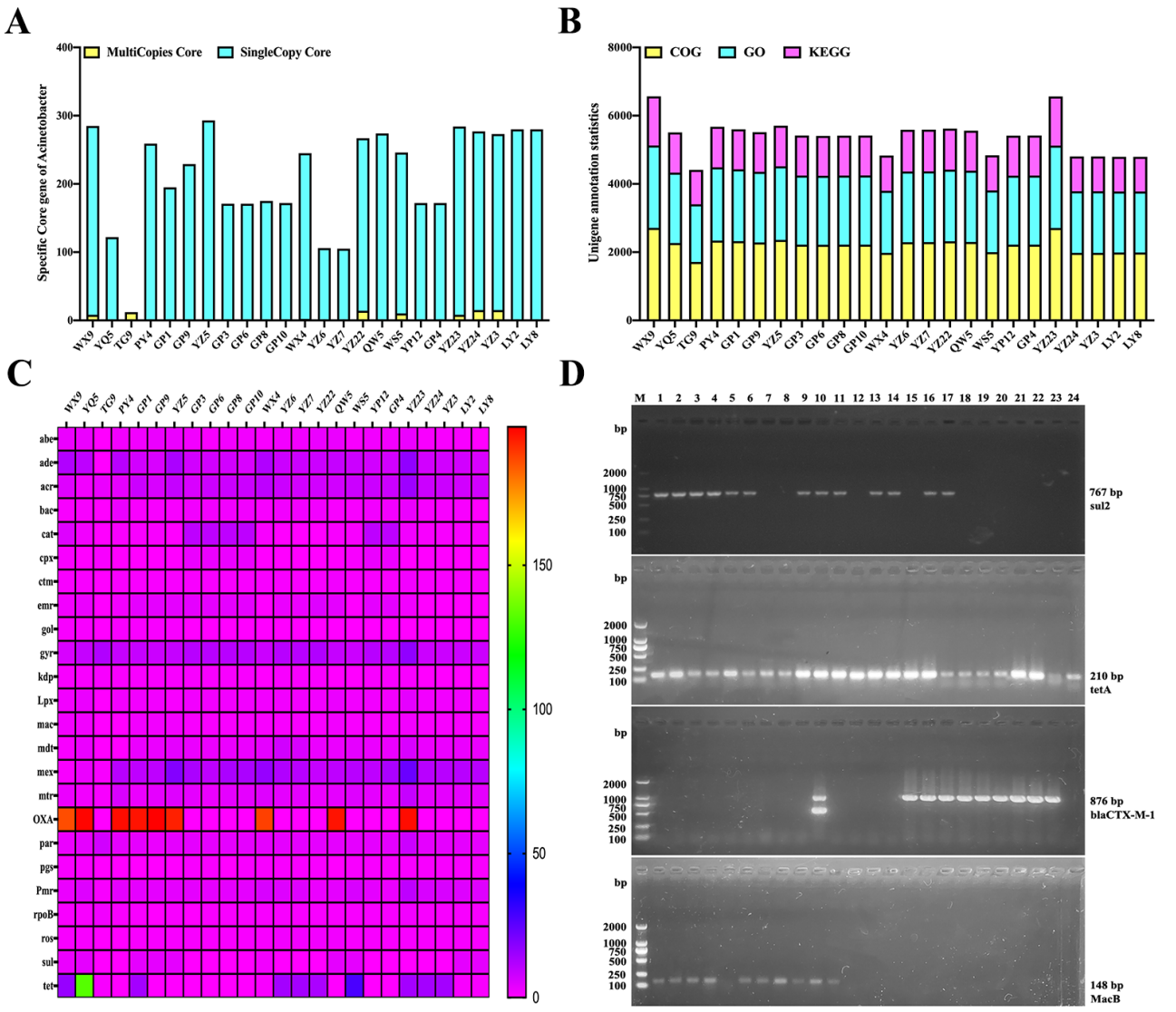

Fig. 1: Core-genome and antibiotic resistance genes analysis in *Acinetobacter*. (A) The diagram showed the number of unique multi-copies core genes and single-copy core genes as a function of the number of genomes among 24 *Acinetobacter* strains. (B) The unigene annotation statistics of 24 *Acinetobacter* strains were based on the COG, GO and KEGG databases. (C) Heatmap of resistance gene cluster in *Acinetobacter* isolates. The different color regions represented the number of genes existing in strains. (D) The expression of *sul2*, *tetA*, $bla_{CTX\text{-}M\text{-}1}$ and *MacB* were detected by PCR. M: DNA maker, 1 to 24 represent WX9, YQ5, TG9, PY4, GP1, GP9, YZ5, GP3, GP6, GP8, GP10, WX4, YZ6, YZ7, YZ22, QW5, WS5, YP12, GP4, YZ23, YZ24, YZ3, LY2 and LY8 respectively.

elevated *MacB* and *TEM* gene expression (Figure. 3M), and both genes were significantly induced at 50μg/mL concentrations of cefotaxime and sulfamethoxazole (Figures. 3N and O). Collectively, these findings affirm the central role of β-lactamase and efflux pump-specific genes in *Acinetobacter spp.* isolates subjected to different antibiotic treatments.

DISCUSSION

Acinetobacter spp., widely prevalent in clinical settings and animal-derived food products, has become infamous for its accumulating antibiotic-resistance genes (Visca et al., 2011). In this study, we sequenced the genomes of 24 *Acinetobacter* isolates, providing valuable insights into their genetic makeup and biotechnological potential. By conducting antibiotic resistance assays and the detection of resistance genes, we evaluated the high rate of MDR and associated resistance gene in these strains. The aim of our research is to facilitate the development of novel drugs and vaccines, thereby enhancing our ability to control and prevent *Acinetobacter* infections in animal populations.

Advances in bacterial genomics have significantly enhanced our understanding of micro diversification within these bacteria. Recent studies utilizing high-throughput sequencing have shed light on epidemiological and evolutionary dynamics within *Acinetobacter* species, revealing the spread of multidrug-resistant clones, the rise of virulent strains, and their evolution within hosts (Antunes et al., 2014; Harris et al., 2010). By employing end-gap free global alignment, we constructed core genomes for our isolated *Acinetobacter* strains. The majority did not exhibit multicopy core genes, except strains WX9, TG9, YZ22, WS5, YZ23, YZ24, and YZ3. The number of specific core genes varied across strains, ranging from 105 to 293, with TG9 being an outlier. The compiled genomic data provides a unique and

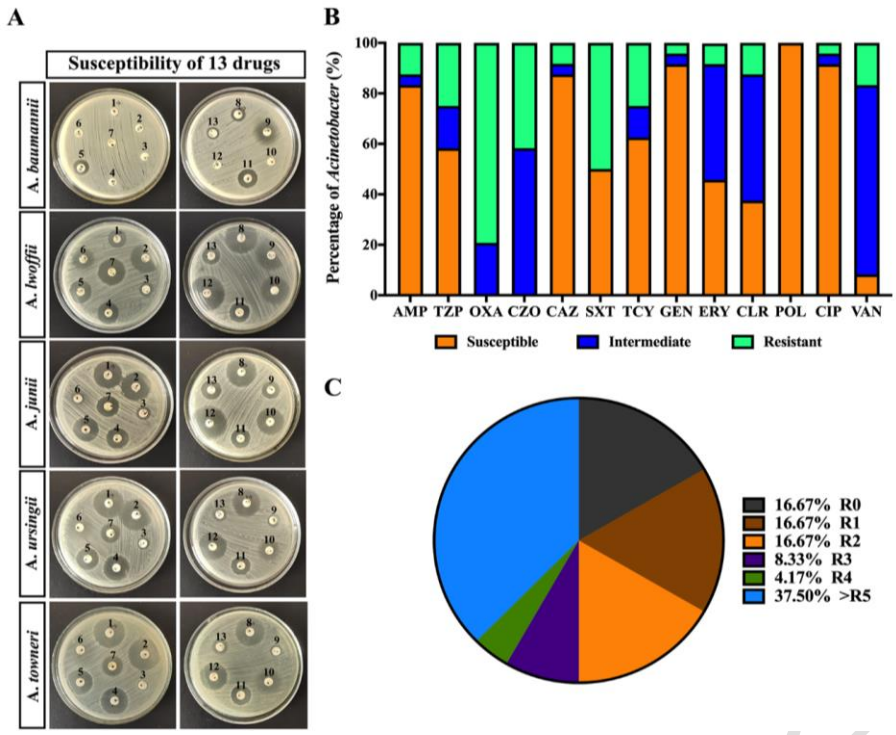

Fig. 2: Antimicrobial resistance rates and multidrug-resistant phenotypes in *Acinetobacter* isolates. (A) The represented MIC plate results of certain isolates displayed resistance rates against used antibiotics in the test. 1 to 13 represent the following concentrations: ampicillin (AMP, 10µg/mL), piperacillin (TZP, 36µg/mL), oxacillin (OXA, 30µg/mL), cefazolin (CZO, 30µg/mL), ceftazidime (CAZ, 30µg/mL), cotrimoxazole (SXT, 25µg/mL), tetracycline (TCY, 6µg/mL), gentamicin (GEN, 10µg/mL), erythromycin (ERY, 30µg/mL), clindamycin (CLR, 2µg/mL), polymyxin B (POL, 2µg/mL), ciprofloxacin (CIP, 2µg/mL), and vancomycin (VAN, 6µg/mL). (B) Resistance rate of isolates to different antibiotics. (C) Multidrug resistance pattern of all isolates. R0: susceptible, R1: resistant to one class of antibiotics, R2: resistant to two classes of antibiotics, R3: resistant to three classes of antibiotics, R4: resistant to four classes of antibiotics, R5: resistant to five classes of antibiotics.

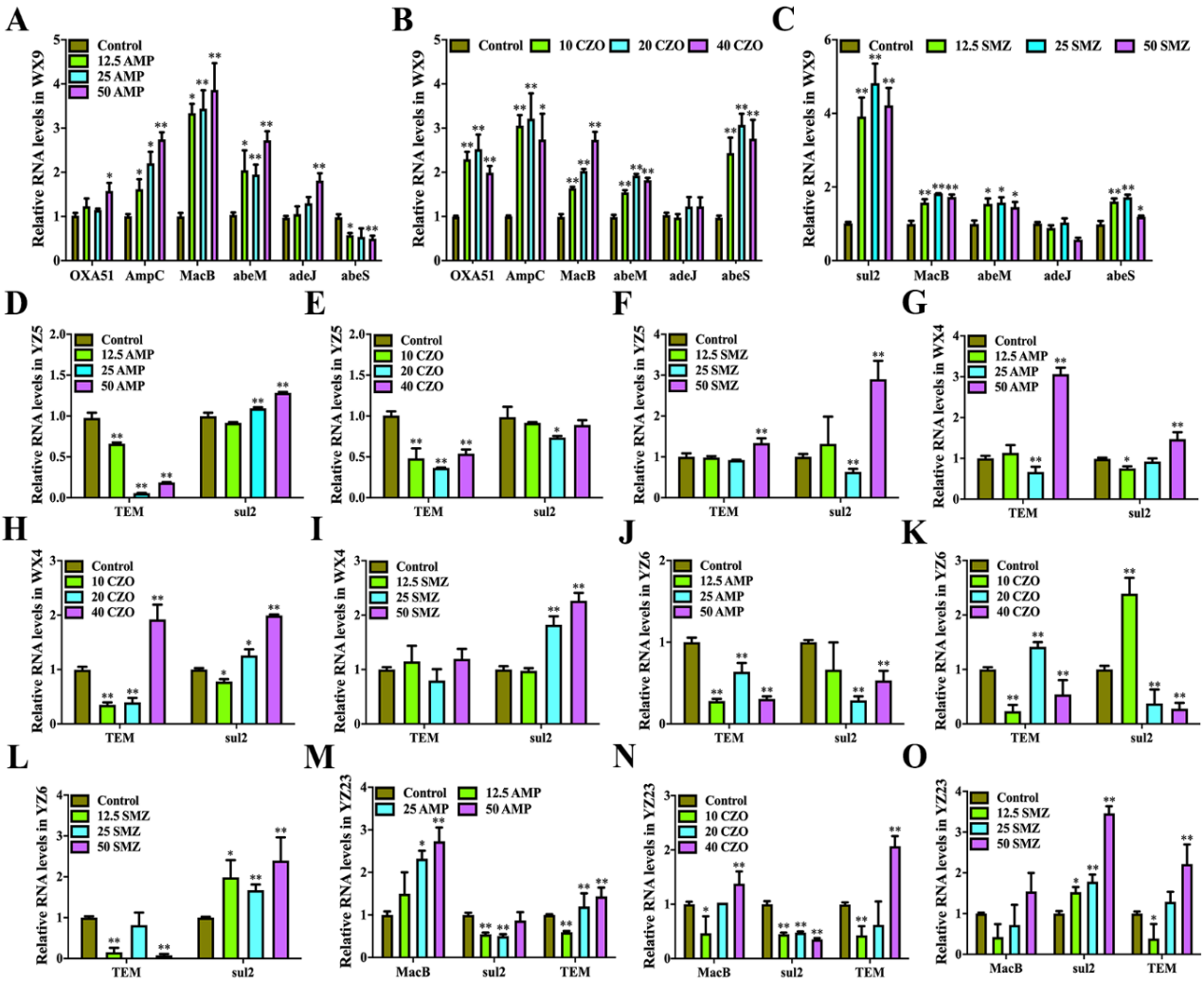

Fig. 3: Resistance gene expression in response to different antibiotics in *Acinetobacter* isolates. The expression of antibiotic resistance genes was detected in certain isolates treated with 0, 12.5, 25, 50µg/mL of ampicillin, cefotaxime, and sulfamethoxazole. (A-C) The expression of *OXA51*, *AmpC*, *MacB*, *abeM*, *adeJ* and *abeS* genes were measured in antibiotics treated-WX9 strain. (D-L) The expression of *TEM* and *sul2* genes were measured in antibiotics treated-YZ5, -WX4, and -YZ6 strains. (M-O) The expression of *MacB*, *TEM*, and *sul2* genes were measured in antibiotics treated-YZ23 strain. Different letters between bars mean $P \leq 0.05$ analyses followed by non-paired Student's t-test. *$P < 0.05$, **$P < 0.01$ vs. Control.

comprehensive reference for high-quality genomes, aiding in the differentiation of *Acinetobacter* species including *A. baumannii*, *A. lwoffii*, *A. junii*, *A. ursingii*, and *A. towneri*. Notably, the content of core genes exhibits significant variability across different species, reflecting a complex interplay with various biological factors including environmental adaptation, gene expression, and the functional aspects of DNA and protein sequences (Teng et al., 2023).

The incidence of multidrug-resistant *Acinetobacter* infections in animals has risen sharply, which poses risks for human colonization and environmental contamination, highlighting One Health concerns (Chen et al., 2021; Wen et al., 2020). Notably, 37.5% of the isolates harbored β-lactam resistance genes *OXA*, including *OXA23*, *OXA24*, *OXA51*, and *OXA58*. Additionally, sulfonamide resistance genes *sul1*, *sul2*, and *sul3* were present in 41.6% of strains, while tetracycline resistance genes *tetA*, *tetB*, and *tetD* were found in 62.5% of isolates. Efflux pump genes *abe* and *ade* were identified in all isolates, with mac present in 91.6%. *Acinetobacter spp.* exhibit characteristic resistance patterns, with intrinsic resistance to agents such as penicillin, ampicillin, amoxicillin, cefazolin, cefuroxime, vancomycin, rifampicin, trimethoprim, and chloramphenicol, as noted in the CLSI guidelines. Intermediate resistance was observed in isolates such as *A. junii* WX4 (50%), *A. ursingii* YZ6 (53.8%), and *A. towneri* YZ23 (53.8%). All isolates showed resistance to antibiotics including ampicillin (12.5%), piperacillin (25%), oxacillin (79.2%), cefazolin (41.7%), ceftazidime (8.3%), cotrimoxazole (50%), tetracycline (25%), gentamicin (4.2%), erythromycin (8.2%), clindamycin (12.5%), polymyxin B (0%), ciprofloxacin (4.2%), and vancomycin (16.7%). The results demonstrated that most isolates showed a high level of multidrug resistance to various antibiotics. The widespread use of sulfonamides, tetracyclines, and aminoglycosides in clinical settings has led to varying levels of resistance in *Acinetobacter* (McCarthy et al., 2021). These findings suggest that the use of benzylpenicillin and sulfonamides should be carefully reconsidered in the management of *Acinetobacter* infections.

In the context of antibiotic resistance, bacteria can also shield targets via genetic mutations or post-translational modifications, or directly neutralize antibiotics through hydrolysis or modification (Blair et al., 2015). Our findings indicate a significant prevalence (41.6%) of the $bla_{CTX\text{-}M\text{-}1}$ gene, suggesting a dominance of $bla_{CTX\text{-}M\text{-}1}$ mediated resistance to β-lactam antibiotics in this region. Tetracycline resistance in Gram-negative bacteria is often linked to the *tetA* and *tetB* genes (Mapipa et al., 2022), and our study confirms the presence of *tetA* gene in all *Acinetobacter* strains, reflecting comprehensive resistance mechanisms against tetracycline, which may involve ribosomal protection, biofilm formation, or efflux pumps. For sulfonamide resistance, the *sul2* gene was most frequently detected (54.1%), pointing to a prevalent *sul2* gene-mediated resistance mechanism in this area. Culturing resistant *Acinetobacter spp.* in various concentrations of sulfamethoxazole consistently resulted in dominant *sul2* gene expression, corroborating its primary role in sulfonamide resistance identified in this study. Conversely, the dominant resistance genes in *Acinetobacter* species under ampicillin or cefotaxime influence were not consistent, hinting at the impact of antibiotic concentration and culture duration, or potentially other unknown resistance mechanisms. Previous research has linked the upregulation of *MacB* gene to *A. baumannii* resistance to tigecycline (Song et al., 2020). Yet, under ampicillin treatment, both *A. baumannii* and *A. towneri* strains predominantly expressed the *MacB* mRNA, suggesting a non-specific action of efflux pumps against antimicrobials. This leads to the hypothesis that additional regulatory mechanisms might influence *MacB* expression in the presence of antimicrobial agents.

In conclusion, this study reveals the characteristics of genomic and drug-resistant genes in isolated *Acinetobacter*, and highlights the high rate of multi-drug resistance, which is predominantly regulated by β-lactamase and efflux pump-associated genes. These findings improve our understanding of antibiotic resistance in foodborne *Acinetobacter*. It provides valuable theoretical insights on clinical breakpoints for susceptibility assay in animal-associated *Acinetobacter* isolates.

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