Research Article

Cell Conflux

Analysis of the Correlation between Pseudomonas aeruginosa Resistance Genes and Virulence Genes in Burn Patients Using Next-Generation Sequencing

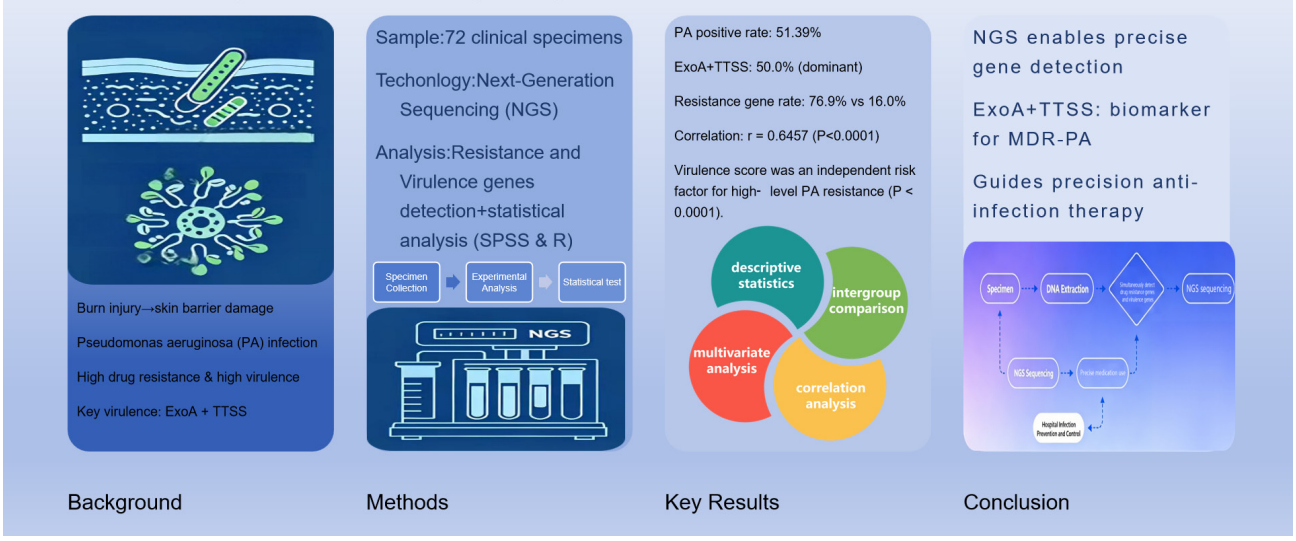
Authors

Zijian Zhang, Delin Hu

Correspondence

yfy128355@fy.ahmu.edu.cn (D. Hu)

Graphical Abstract



https://doi.org/10.71321/dpqzt488

Research Article

Cell Conflux

Analysis of the Correlation between Pseudomonas aeruginosa Resistance Genes and Virulence Genes in Burn Patients Using Next-Generation Sequencing

Zijian Zhang [1], Delin Hu [1*]

Received: 2026-04-23 | Accepted: 2026-05-25 | Published online: 2026-07-14

Abstract

Objective: To analyze the distribution and correlation of drug resistance genes and virulence genes of Pseudomonas aeruginosa (PA) isolated from burn patients using next-generation sequencing (NGS), providing molecular evidence for precise clinical diagnosis and treatment.

Methods: A retrospective study was conducted on 72 suspected PA infection specimens and clinical data collected from the Department of Burn and Wound Repair Surgery at the First Affiliated Hospital of Anhui Medical University between January 2022 and December 2025. NGS was employed to detect drug resistance and virulence genes, with statistical analysis performed using SPSS 26.0 and R language.

Results: The positive detection rate of PA was 51.39% (37/72), with wounds being the primary infection site (positive rate: 83.8%). The infection rate was highest among moderate burn patients (58.3%). PA exhibited a 100.0% resistance rate to cefazolin, and among the 25 drug-sensitive strains, 36.0% were multidrug-resistant. A trend toward prevalence of PA resistant to carbapenems was observed. Among the 52 specimens tested for virulence genes, the overall carriage rate was 51.9% (27/52), with the ExoA+TTSS composite genotype being predominant (50.0%, 26/52). The detection rate of drug resistance genes in ExoA+TTSS-positive strains (76.9%) was significantly higher than in non-virulence gene-positive strains (16.0%; χ^2=19.9086, P<0.0001). The virulence factor score showed a moderately strong positive correlation with the number of drug resistance genes (r=0.6457, P<0.0001) and was identified as an independent risk factor for drug resistance gene carriage (OR=2.1494–9.7401, P<0.0001).

Conclusion: In burn patients, PA infections predominantly occur at the wound site, with prominent resistance and multidrug resistance phenomena; PA virulence genes exhibit a significant positive correlation with resistance genes, and strains with the ExoA+TTSS composite genotype demonstrate higher resistance; NGS enables simultaneous analysis of PA genetic characteristics, providing critical support for precision anti-infection therapy.

Keywords: Burn; Pseudomonas aeruginosa (PA); Next-generation sequencing (NGS); Drug resistance genes; Virulence genes

Introduction

Burns compromise the skin barrier and induce systemic immune dysfunction, rendering patients highly susceptible to invasive infections. Pseudomonas aeruginosa (PA) is the predominant opportunistic pathogen causing burn wound infections, frequently leading to severe sepsis, multiple organ dysfunction syndrome, and even patient mortality [1]. With the deepening of burn care and the wide application of broad-spectrum antibiotics, the clinical prevention and control of PA infections have become increasingly challenging. The clinical diagnosis and treatment of Pseudomonas aeruginosa infections present significant challenges, as the bacterial strains exhibit multidrug resistance (MDR) and high virulence. Particularly, the prevalence of carbapenem-resistant Pseudomonas aeruginosa (CRPA) has become a central therapeutic challenge in global burn care [2]. In recent three years, the detection rate of CRPA in domestic burn wards has reached 45%–65%, showing a continuous upward trend, which seriously restricts the efficacy of clinical anti-infection treatment.

The pathogenicity of Pseudomonas aeruginosa is strictly regulated by virulence genes, among which exotoxin A (ExoA) and the type III secretion system (TTSS) serve as core virulence factors mediating host cell damage and bacterial dissemination. Diverse resistance genes confer resistance to β-lactams, aminoglycosides, quinolones, and carbapenems through mechanisms such as enzyme inactivation, overexpression of efflux pumps, and modification of target sites. Notably, an increasing number of clinical studies have indicated a potential synergistic relationship between virulence and resistance in clinically isolated PA strains. However, specialized studies investigating this correlation in burn patients—a population with unique wound microenvironments and immune states—remain scarce.

1 Department of Burn and Wound Repair Surgery, The First Affiliated Hospital of Anhui Medical University, Hefei, China.

* Corresponding Author.

Next-generation sequencing (NGS) enables high-throughput, simultaneous detection of bacterial resistance genes and virulence genes, demonstrating significantly superior accuracy and comprehensiveness compared to traditional drug susceptibility testing and single-gene PCR detection techniques [3]. At present, most domestic and foreign studies focus on the distribution of single type of genes or the correlation analysis in common clinical populations, while there is a lack of systematic research on the co-distribution and molecular correlation of resistance and virulence genes of PA in burn patients, and the exploration of synergistic mechanisms is also insufficient. To investigate the relevant experiments, this study employed NGS technology to analyze the distribution patterns of Pseudomonas aeruginosa resistance genes and virulence genes in burn patients and to investigate their correlation. The findings aim to provide a molecular biological basis for the precise diagnosis, individualized antimicrobial therapy, and targeted infection prevention and control of Pseudomonas aeruginosa infections in burn patients.

Materials and Methods

Study Subjects

A retrospective study was conducted on 72 specimens from patients suspected of PA infection admitted to the Department of Burn and Wound Repair Surgery at the First Affiliated Hospital of Anhui Medical University between January 2022 and December 2025, including wound tissue, blood, sputum, and central venous catheter specimens. Clinical data such as patient gender, age, burn area, burn depth, and infection site were collected.

Inclusion and Exclusion Criteria

Inclusion criteria: The strain was confirmed as PA through morphological examination, biochemical tests, VITEK MS mass spectrometry, and 16S rDNA sequencing; the strain was isolated from clinical specimens of burn patients with a purity >99% confirmed by subculture; the patient's clinical data were complete; the infection was solely due to burns; and no novel antimicrobial agents such as cefotaxime/avibactam or meropenem/flobopenem were administered prior to strain isolation. Exclusion criteria: Contaminated strains or mixed infection strains; absence of critical clinical data; presence of severe underlying conditions such as malignant tumors or multiple organ dysfunction syndrome; homologous strains isolated repeatedly from the same patient during hospitalization (confirmed by whole-genome homology analysis).

Detection Method

NGS detection was performed using the Illumina MiSeq platform and supporting Nextera XT DNA Library Preparation Kit (Illumina Inc., Model: FC-131-1096). The detection of PA resistance genes (β-lactamase genes, aminoglycoside-modifying enzyme genes, fluoroquinolone resistance genes) and virulence genes (ExoA, TTSS) was performed strictly following the kit instructions.

Statistical Analysis

Data analysis was performed using SPSS 26.0 and R 4.2.1 software. Categorical data were expressed as counts (%). Intergroup comparisons were analyzed using the χ^2 test or Fisher's exact probability method; correlation was assessed with Spearman's rank correlation analysis; and multivariate analysis was conducted using logistic regression analysis. A P-value <0.05 was considered statistically significant.

Results

Clinical Distribution Characteristics of PA

Among the 72 specimens, PA positivity was detected in 37 cases, yielding a detection rate of 51.39% (37/72). The majority of specimens (56 cases, 77.8%) were wound tissues, with a PA positivity rate of 83.8% (31/37) (Figure 1). Among the 45 burn patients, those with moderate burns (body surface area 10%–30%) exhibited the highest PA infection rate (58.3%), as detailed in Table 1.

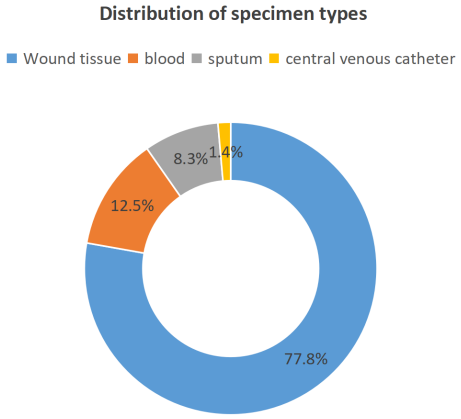


Figure 1. Distribution of specimen types.
The pie chart displays the proportion of different specimen types. Wound tissue accounts for 77.8%, blood 12.5%, sputum 8.3%, and central venous catheter 1.4%.

Table 1. Summary of Descriptive Statistics for Pseudomonas aeruginosa Detection Samples.

project	quantity	percentage (%)
Total number of specimens	**72**	**100.0**
Wound tissue	56	77.8
blood	9	12.5
sputum	6	8.3
central venous catheter	1	1.4
Burn patient	45	62.5
Non-burn patients	27	37.5
Mild burn (<10%)	2	2.8
Moderate burns (10%–30%)	14	9.4
Severe burns (>30%)	29	40.3
Resistant gene detected	35	48.6
No drug-resistant genes were detected.	37	51.4
Virulence factors were detected	27	51.9
No virulence factors were detected.	25	48.1

Note: This table summarizes the descriptive statistics for 72 Pseudomonas aeruginosa detection samples, covering specimen type composition, burn/non-burn patient distribution, burn severity grading, and detection proportions of drug-resistant genes and virulence factors.

PA Resistance Characteristics

Among the 25 strains with complete antibiotic susceptibility profiles, the resistance rates of PA were 100.0% to cefazolin, 83.3% to gentamicin, 77.8% to ciprofloxacin, 75.0% to meropenem, and 72.7% to cefoperazone-sulbactam (Figure 2). Multidrug-resistant strains accounted for 36.0% (9/25), with one highly resistant strain exhibiting resistance to 11 antimicrobial agents. A total of 24 resistance genes were identified, among which the combination of FosA, OXA-50, APH(3')-IIb, and catB7 showed the highest detection rate (8.1%, 3/37).

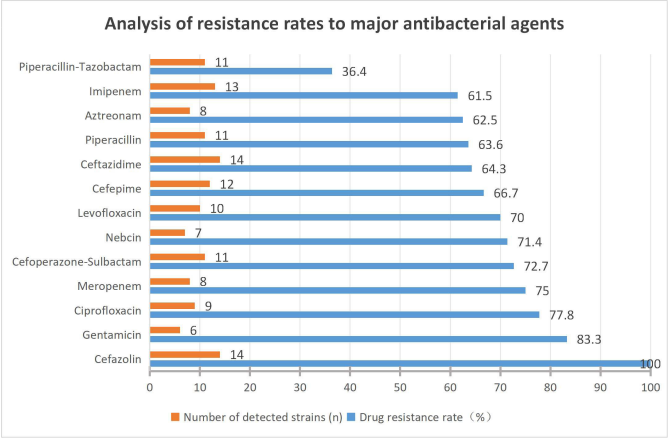


Figure 2. Analysis of resistance rates to major antibacterial agents. The bar chart shows PA resistance rates to common antibiotics: cefazolin 100.0%, gentamicin 83.3%, ciprofloxacin 77.8%, meropenem 75.0%, cefoperazone-sulbactam 72.7%.

Distribution Characteristics of PA Virulence Genes

Among the 52 specimens for which virulence gene testing was completed, the overall carriage rate of virulence genes was 51.9% (27/52); the detection rate of the ExoA+TTSS composite genotype was 50.0% (26/52), the detection rate of TTSS alone was 1.9% (1/52), and no virulence genes were detected in 48.1% (25/52) of the specimens (Figure 3). The detection rates of virulence genes across different specimen types are presented in Table 2.

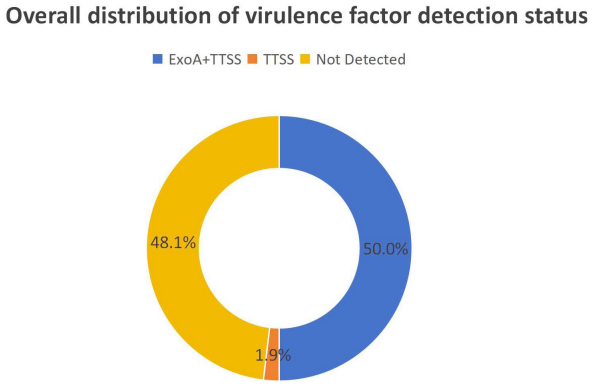


Figure 3. Overall distribution of virulence factor detection status. The chart shows PA virulence gene carriage rate: total 51.9%, ExoA+TTSS 50.0%, TTSS alone 1.9%, no virulence genes 48.1%.

Correlation between virulence genes and drug resistance genes

The detection rate of drug resistance genes in ExoA+TTSS co-positive strains was 76.9% (20/26), significantly higher than that in non-virulence gene strains (16.0%, 4/25), with a statistically significant difference (χ^2=19.9086, P<0.0001) (Table 3). This study established a virulence factor scoring system to classify samples into three groups: Group 0: no relevant virulence genes detected; Group 1: only the virulence gene TTSS detected; Group 2: both ExoA and TTSS detected. A count-based indicator for the number of drug resistance genes was constructed, measuring the total number of drug resistance gene types carried by each strain. The virulence factor score showed a moderately strong positive correlation with the number of drug resistance genes (r=0.6457, P<0.0001) (Figure 4). Logistic regression analysis demonstrated that the virulence factor score was an independent risk factor for the presence of PA drug resistance genes (OR=2.1494–9.7401, P<0.0001).

Table 2. Detection rates of virulence genes in various specimen types.

Specimen Type	Total sample size	Virovirulence gene positive	Viroviru-lence gene negative	relevance ratio (%)
blood	9	0	9	0.00
central venous catheter	1	0	1	0.00
sputum	5	5	0	100.00
Wound tissue	37	22	15	59.46
Overall	52	27	25	51.92

Note: This table shows the detection rates of virulence genes in 52 valid samples across different specimen types (blood, central venous catheter, sputum, wound tissue), as well as the overall virulence gene detection rate.

Table 3. Presence rates of drug resistance genes in strains with different virulence factor types.

Type of virulence factor	Resistant gene-positive	Negative for drug resistance genes	amount to	relevance ratio (%)
ExoA+TTSS complex-positive	20	6	26	76.9
No virulence factors were detected.	4	21	25	16.0
TTSS alone is positive	0	1	1	0.0
amount to	24	28	52	-

Note: This table presents the detection rates of drug resistance genes in 52 Pseudomonas aeruginosa strains with distinct virulence factor types, illustrating the relationship between virulence factor profiles and drug resistance gene carriage.

Discussion

Clinical Distribution Characteristics of Pseudomonas aeruginosa in Burn Patients

Among the 72 suspected infection specimens in this study, the positive detection rate of Pseudomonas aeruginosa was 51.39%, consistent with epidemiological surveillance data

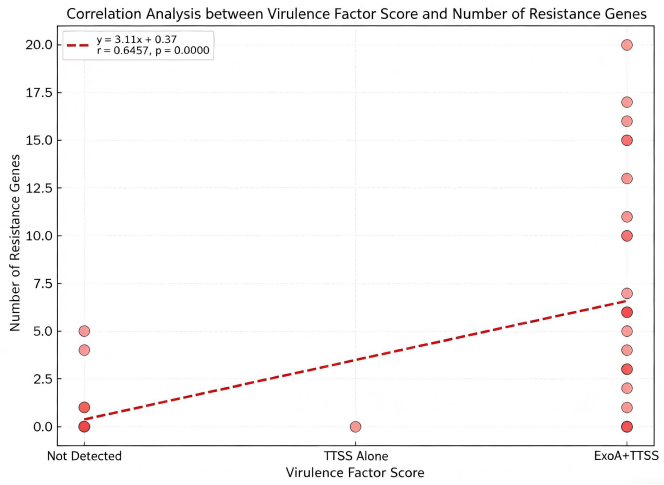


Figure 4. Correlation Analysis between Virulence Factor Score and Antibiotic Resistance Gene Number.

The scatter plot shows a moderately strong positive correlation (r=0.6457, P<0.0001), regression equation y=3.14x+0.37.

from major domestic burn treatment centers [4]. Wound tissue specimens accounted for 77.8% of the total specimens, with a Pseudomonas aeruginosa positivity rate of 83.8%, confirming that burn wounds are the primary site for bacterial colonization and infection. This finding is attributed to the loss of skin barrier integrity post-burn, the nutrient-rich exudate from wounds, and impaired local immune function, which collectively create an optimal microenvironment for Pseudomonas aeruginosa proliferation [5]. Notably, moderate burn patients (total burn area 10%–30%) exhibited the highest Pseudomonas aeruginosa infection rate (58.3%), likely correlating with the balance between wound healing progress, host immune recovery status, and the selective pressure exerted by antimicrobial agents during this disease stage [6].

Drug Resistance Characteristics of Pseudomonas aeruginosa Strains

In this study cohort, Pseudomonas aeruginosa exhibited significant resistance to first-line antimicrobial agents, with a cephalosporin resistance rate of 100.0%, as well as high resistance rates to gentamicin (83.3%), ciprofloxacin (77.8%), and meropenem (75.0%). Among the 25 strains with complete susceptibility testing results, 36.0% were multidrug-resistant strains, with one strain demonstrating broad resistance to 11 antimicrobial agents. These findings align with the 2023–2024 monitoring report from the China Antimicrobial Resistance Surveillance Network (CARST), highlighting the severe prevalence of multidrug-resistant and carbapenem-resistant Pseudomonas aeruginosa in burn and trauma wards [7]. A total of 24 drug resistance genes were identified in this study, with the FosA/OXA-50/APH(3')-IIb-catB7 gene combination showing the highest detection rate (8.1%). The excessive and irrational use of broad-spectrum antimicrobial agents in burn and trauma wards are the primary drivers of the acquisition and horizontal transmission of such resistance genes [8].

Distribution Characteristics of Virulence Genes

Among the 52 specimens with completed virulence gene testing, the overall carriage rate of virulence genes was 51.9%. The ExoA+TTSS composite genotype was the predominant genotype (50.0%), and no strains carrying the ExoA gene alone were identified. These results indicate that the secretion and cytotoxic activity of ExoA are mediated by the TTSS system, which directly transports virulence effector proteins into host cells for their functions [9]. The positivity rate of virulence genes in respiratory specimens reached 100.0%, suggesting that Pseudomonas aeruginosa colonizing the respiratory tract exhibits stronger virulence and a higher propensity for systemic disseminated infections, necessitating enhanced clinical surveillance and prevention measures [10]. This finding has important clinical practical significance: for burn patients complicated with respiratory infection, clinical staff should prioritize rapid screening of PA virulence genes in respiratory specimens. Patients with positive results need timely single-room isolation and enhanced respiratory tract protection to avoid nosocomial cross-infection. Meanwhile, individualized anti-infective regimens covering highly virulent PA should be formulated early to reduce the risk of systemic infection and improve treatment efficacy.

Statistical analysis of the correlation between virulence genes and drug resistance genes

The correlation analysis of this study further clarified the close link between PA virulence and resistance genes in burn patients. It suggested an extremely significant association between virulence genotypes and drug resistance genotypes: the detection rate of drug resistance genes in ExoA+TTSS-positive strains (76.9%) was significantly higher than that in non-virulence gene-positive strains (16.0%; χ^2=19.9086, P<0.0001). Spearman's correlation analysis confirmed a moderately strong positive correlation between virulence factor scores and the number of drug resistance genes (r=0.6457, P<0.0001). Multivariate logistic regression analysis further demonstrated that virulence factor scores were an independent risk factor for drug resistance gene carriage in strains (OR=2.1494–9.7401, P<0.0001).

The molecular mechanisms underlying their synergistic interaction primarily include three aspects:

1.Quorum sensing (QS) system-mediated regulation of cross-linking: The Las/Rhl-type QS system synchronously activates the transcription of virulence genes (e.g., exoA, ttss) and efflux pump resistance genes (e.g., mexAB-oprM), thereby concurrently enhancing bacterial virulence and drug resistance [11];

2.Mediation by mobile genetic elements (MGEs): Plasmids, integrons, and transposons can simultaneously carry both virulence and resistance genes, facilitating horizontal gene transfer and co-screening under antimicrobial selection pressure [12];

3.Selective effects of the wound microenvironment: Highly virulent Pseudomonas aeruginosa exhibits stronger adhesion and invasion capabilities, prolonged colonization duration in hosts, higher exposure to antimicrobial agents, and greater susceptibility to acquiring resistance genes [13].

In addition, the hypoxic and highly inflammatory microenvironment of burn wounds can induce PA stress response, upregulate the activity of the QS system, and further amplify the synergistic effect of resistance and virulence, increasing the difficulty of clinical treatment.

Research Limitations and Clinical Implications

This study has certain limitations: First, it is a single-center retrospective study with a relatively small sample size, which may introduce selection bias and limit the generalizability of the findings; second, virulence gene detection only covered exoA and ttss, excluding adhesion-related (pilA, algD) and protease-related (lasB, aprA) virulence genes, potentially overlooking other virulence-resistance regulatory pathways; finally, no in vitro functional validation experiments (e.g., gene knockout, transcriptomic analysis) were conducted, making it impossible to directly elucidate the molecular regulatory mechanisms underlying the association between these factors.

Despite the aforementioned limitations, the findings of this study still hold significant clinical value: NGS technology enables rapid and simultaneous analysis of the drug resistance and virulence gene profiles of Pseudomonas aeruginosa, providing support for the precise management of antimicrobial therapy in burn patients; the ExoA+TTSS genotype can serve as a molecular biomarker for identifying high-risk multidrug-resistant Pseudomonas aeruginosa infections, guiding early clinical intervention and infection control. Clinically, this biomarker can be used for routine screening of PA isolates from burn patients. For ExoA+TTSS-positive strains, clinicians should avoid using highly resistant antibiotics such as cephalosporins, and prioritize selecting sensitive antibiotics combined with anti-virulence adjuvant therapy to optimize individualized treatment plans and reduce the risk of nosocomial infection spread. Future large-scale, multicenter prospective studies combined with functional genomics experiments are required to elucidate the molecular mechanisms underlying the virulence-resistance coupling in Pseudomonas aeruginosa, thereby facilitating the development of targeted therapeutic strategies for burn-associated Pseudomonas aeruginosa infections.

Conclusion

In burn patients, PA infections are centered around the wound site, with severe bacterial resistance and multidrug resistance phenomena; the PA ExoA+TTSS composite virulence genes exhibit a significant positive correlation with resistance genes, markedly increasing the risk of resistance in highly virulent strains [14]. NGS technology enables simultaneous and precise detection of PA resistance and virulence genes, providing critical molecular evidence for the accurate diagnosis of PA infections in burn patients, personalized selection of antimicrobial agents, and hospital infection prevention and control.

Abbreviations

CARST: National Bacterial Resistance Surveillance Network; CRPA: carbapenem-resistant Pseudomonas aeruginosa; ExoA: exotoxin A; NGS: next-generation sequencing; PA: Pseudomonas aeruginosa; TTSS: type III secretion system.

Author Contributions

Zijian Zhang: Conceptualization, Methodology, Data curation, Writing – original draft, Investigation, Validation, Formal analysis. Delin Hu: Supervision, Writing – review & editing, Project administration.

Acknowledgments

Not applicable.

Funding Information

Not applicable.

Ethical Approval and Consent to Participate

Not applicable.

Competing Interests

The authors declare that they have no known competing financial or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

All data supporting the conclusions of this article are included within the article. The authors may be contacted for additional data related to this paper if required.

References

[1] Yao Yongming, Ren Chao. Burn Infection and Immune Disorders: A New Perspect-ive in Translational Medicine from Pathogenesis to Precision Diagnosis and Treat-ment[J]. Chinese Journal of Burn and Wound Care, 2026, 42(2): 119-125. https://doi.org/10.3760/cma.j.cn501225-20251104-00457.

[2] Li Suli, Hua Chuan. Research Progress on Multidrug Resistance and Pan-Resistan-ce of Pseudomonas aeruginosa[J]. Shanxi Medical Journal (Second Half Monthly Issue), 2013, 42(8): 891-892.

[3] Huang Jingjing, Xiao Meng, Xu Yingchun.Application of next-generation sequencing technology in microbiology and infectious diseases[J]. Journal of Union Medical College, 2018, 9(5): 5. https://doi.org/10.3969/j.issn.1674-9081.2018.05.014.

[4] Hoque M , Jahan M, Hossain M A, et al. Genomic diversity and molecular epide-miology of a multidrug resistant Pseudomonas aeruginosa DMC30b isolated from hospitalized burn patient in Bangladesh[J]. bioRxiv, 2022. https://doi.org/10.1101/2022.07.06.498939.

[5] Wang Lingshuang. Application of mussel-inspired adhesive hydrogel in wound repair [D]. Southwest University, 2024.

[6] Wei Quanzhen, Zhang Huizhen, Liu Lihua. Prevention and control of MRSA hospit-al-acquired infections in wound surfaces of burn and trauma patients[J]. Chinese

Journal of Hospital Infection, 2007,17(7): 3. https://doi.org/10.3321/j.issn:1005-4529.2007.07.022.
[7] Aroca Molina K J, Gutiérrez S J, Benítez-Campo N, et al. Genomic Differences Associated with Resistance and Virulence in Pseudomonas aeruginosa Isolates from Clinical and Environmental Sites[J]. Microorganisms, 2024, 12(6). https://doi.org/10.3390/microorganisms12061116.
[8] Bi Wenzhi, Zhou Tieli. Research progress on bacterial pathogenicity, current status of drug resistance, and mechanisms of resistance [J]. Zhejiang Medical Journal, 2018,40(20):5. https://doi.org/10.12056/j.issn.1006-2785.2018.40.20.2018-1548
[9] MartínezE, Im H, Mohasin M, et al. The Oxylipin Dependent Quorum Sensing Sys-tem enhances Pseudomonas aeruginosa dissemination during burn-associated infe-ction[J]. PLoS Pathogens, 2026, 22(1). https://doi.org/10.1371/journal.ppat.1013885.
[10] Reid O. Burn wound infections[J]. Clinical Microbiology Reviews, 2006, 19(2): 403-434. https://doi.org/10.1128/cmr.19.2.403-434.2006.
[11] Zhou Kai, Shi Cuixiao, Song GuoBin, Huang Ying,Xu Yuanhong. Carriage, expres-sion, and correlation with drug resistance of QS system regulatory genes and viru-lence genes in Pseudomonas aeruginosa[D]. Hefei: Anhui Medical University, 2022. https://doi.org/10.19405/j.cnki.issn1000-1492.2022.05.014.
[12] Weng Xingbi, Mi Zuhuang. Mobile genetic elements: vectors for drug resistance genes[J]. China Journal of Zoonotic Diseases, 2013, 29(4): 9. https://doi.org/10.3969/cjz.j.issn.1002-2694.2013.04.015.
[13] Xu Y, Wang Y, Zhang H, et al. Detection of virulence genes and drug resistance genes in Pseudomonas aeruginosa isolates from elderly patients with acquired pneumonia[J]. Chinese Journal of Clinical Infectious Diseases, 2019. https://doi.org/10.3760/cma.j.issn.1674-2397.2019.01.011.
[14] Zhuo Chao, Wang Luxia, Xiao Shunian, et al. Clinical significance of virulence genes associated with the Pseudomonas aeruginosa type III secretion system[J]. Chinese Journal of Burns, 2010(5): 6. https://doi.org/10.3760/cma.j.issn.1009-2587.2010.05.009.