Research Square

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Colonization, Molecular Epidemiological Characteristics, Antimicrobial Resistance, and Related Risk Factors of Clostridioides difficile in Elderly Hospitalized Patients

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Abstract

Objective: To investigate the colonization status, molecular epidemiological characteristics, antimicrobial resistance profiles, and risk factors for *Clostridioides difficile* (*C. difficile*) colonization in elderly hospitalized patients, and to provide evidence for clinical prevention and control.

Methods: A total of 300 elderly inpatients admitted to geriatric wards between January 2022 and December 2024 were enrolled. Inclusion criteria were age ≥65 years, hospital stay ≥72 h, complete clinical data, and absence of diarrhea with normal stool appearance upon admission. Patients without stool specimens or who refused specimen collection were excluded. Stool specimens were collected for *C. difficile* isolation and identification. The toxin genes tcdA and tcdB were detected by PCR. Multilocus sequence typing (MLST) was performed for molecular epidemiological analysis. The agar dilution method was used to determine minimum inhibitory concentrations (MICs) for nine antimicrobial agents. Clinical data were collected; univariate and multivariate logistic regression analyses were applied to identify risk factors for *C. difficile* colonization.

Results: Among the 300 elderly inpatients, 95 (31.67%) were colonized with C. *difficile*. Of the 95 isolates, 41 (43.16%) were non-toxigenic (tcdA-/tcdB-), and 54 (56.84%) were toxigenic. All toxigenic strains were tcdA+/tcdB+, and no tcdA-/tcdB+ strains were detected. The 95 isolates were assigned to 15 sequence types (STs). ST81 was predominant (28 isolates, 29.47%), followed by ST39 (18 isolates, 18.95%). All isolates were susceptible to vancomycin and metronidazole. Univariate analysis showed that older age, immunocompromised status, longer hospital stay, and antibiotic exposure were significantly associated with *C. difficile* colonization ($P < 0.05$). Multivariate logistic regression confirmed older age, immunocompromised status, prolonged hospital stay, and antibiotic exposure as independent risk factors for *C. difficile* colonization ($P < 0.05$).

Conclusion: The *C. difficile* colonization rate among elderly hospitalized patients is relatively high; ST81 and ST39 as major genotypes, with toxigenic strains accounting for 56.84% of isolates. Older age, immunocompromised status, longer hospital stay, and antibiotic exposure are independent risk factors for *C. difficile* colonization. Vancomycin and metronidazole remain preferred agents for empirical CDI therapy. High resistance rates were observed for cephalosporins, clindamycin, and ciprofloxacin; these agents are not recommended for CDI treatment. Caution should be exercised when extrapolating susceptibility results from asymptomatic colonizing isolates to clinical CDI infections.

1 Introduction

Clostridioides difficile is a Gram-positive, strictly anaerobic, spore-forming bacillus. Spores are transmitted mainly via the fecal–oral route and are widely distributed in the environment. They exhibit strong resistance to ethanol, high temperature, and desiccation, can persist on environmental surfaces for months to years, and are highly transmissible and difficult to eradicate. *C. difficile* pathogenicity is primarily mediated by two glucosylating toxins, toxin A (TcdA) and toxin B (TcdB); a subset of strains additionally produce binary toxin.

Widespread antimicrobial consumption has been accompanied by increasing antimicrobial resistance in *C. difficile* and the emergence of hypervirulent lineages such as NAP1/027/BI, which have caused international outbreaks of *Clostridioides difficile* infection (CDI) with substantial morbidity and mortality. In 2011, nearly 500,000 CDI cases occurred in the United States, with approximately 29,000 deaths within 30 days; persons aged ≥ 65 years had an incidence rate 8.65-fold higher than younger populations[1]. European multicenter surveillance initially linked the surge in CDI incidence and severity to the emergence and rapid dissemination of the hypervirulent RT027 strain, which exhibited fluoroquinolone resistance and enhanced toxin production[2]. However, the epidemiology of CDI has shifted substantially in recent years. Following the implementation of infection control measures and restrictions on fluoroquinolone prescribing, RT027 prevalence declined markedly across North America and Western Europe, and it is now considered to have undergone “apparent eradication” in some national surveillance systems such as Belgium[3]. Despite these advances in understanding the changing epidemiology, CDI surveillance data remain limited across most regions, including the Asia-Pacific. In China, a meta-analysis reported a pooled CDI prevalence of 11.4% among hospitalized patients with diarrhea, with considerable geographic heterogeneity, and large-scale multicenter molecular epidemiological data for C. *difficile* remain scarce[4,5].

CDI is closely linked to antibiotic administration and hospitalization; hospitalized patients constitute a high-risk population[6]. Elderly individuals are particularly vulnerable due to age-related immunosenescence, frequent antibiotic exposure, and repeated healthcare contact[7, 8]. Excessive or inappropriate antibiotic use disrupts beneficial gut microbiota, weakening colonization resistance against C. *difficile*[8]. When drug-resistant C. *difficile* overgrows in elderly hosts, gut dysbiosis and intractable infectious diarrhea may develop[6, 8]. Therefore, characterizing molecular epidemiological features and risk factors of *C. difficile* among elderly populations is critical for clinical prevention and management.

This study screened for *C. difficile* among elderly hospitalized patients in Jiaxing, China. We analyzed colonization rate, toxin-gene profiles, MLST genotypes, and antimicrobial susceptibility, and explored colonization-related risk factors, aiming to provide local evidence for *C. difficile* prevention and control and rational antibiotic prescription.

2 Materials and Methods

2.1 Study Population

Three hundred elderly inpatients admitted to geriatric wards from January 2022 to December 2024 were enrolled: 197 males and 103 females, aged 66–97 years (mean 86.27 ± 9.65 years). Inclusion criteria were: (1) age ≥ 65 years; (2) hospital stay ≥ 72 h; (3) complete clinical records; and (4) no diarrhea and normal stool appearance on admission. Exclusion criteria were absence of stool specimens or refusal to provide stool samples. The study was approved by the hospital ethics committee; informed consent was obtained from patients or their legal surrogates.

2.2 Specimen Collection, Strain Isolation, and Identification

Stool specimens were collected using sterile containers, each specimen at least 3 g. One gram of stool was transferred into a centrifuge tube, mixed with an equal volume of absolute ethanol, vortexed thoroughly, and left to stand for 30 min. After centrifugation at 3500 rpm for 15 min, the supernatant was discarded. The sediment was inoculated onto CDIF selective agar and incubated anaerobically at 35°C for 48 h. Suspected colonies were selected according to colony morphology, Gram-staining features, and characteristic horse-stable odor. Isolates were identified using the VITEK MS IND MALDI-TOF mass-spectrometry system (bioMérieux). Isolates were subcultured for purification and stored in glycerol at −80°C.

2.3 DNA Extraction

One- to two-day-old colonies were suspended in sterile saline to prepare a 0.5 McFarland turbidity suspension. After centrifugation (10,000 rpm, 1 min), the supernatant was discarded; 180 µL lysis buffer plus 20 µL lysozyme (200 mg/mL) were added. The mixture was incubated at 37°C for 30 min. Nucleic acid extraction was performed using a Thermo Fisher automated nucleic-acid extractor according to the manufacturer's protocols.

2.4 Toxin-Gene Detection

Primers targeting tcdA and tcdB were designed according to Kato et al. [9, 10]. The 25-µL PCR system contained 12.5 µL 2× Taq Master Mix, 10 pmol of each primer, 1 µL DNA template, and ddH_2O to 25 µL. The amplification program was: initial denaturation at 94°C for 5 min; 30 cycles (94°C for 40 s; 52°C for 40 s; 72°C for 40 s); and final extension at 72°C for 10 min. PCR products were separated by 1% agarose-gel electrophoresis (110 V, 20–30 min) and visualized with gel-imaging equipment.

2.5 Multilocus Sequence Typing (MLST)

MLST was performed as described by Griffiths et al. [11] using seven housekeeping genes: adk, atpA, dxr, glyA, recA, sodA, and tpi. The 25-µL PCR reaction contained 12.5 µL of 2× Pfu Master Mix, 1 µL each forward/reverse primer (10 µmol/L), 1 µL DNA template, and 9.5 µL ddH_2O. Cycling parameters were: initial 94°C for 15 min; 35 cycles (94°C for 30 s; 50°C for 40 s; 72°C for 70 s); and final extension at 72°C for 5 min. Amplicons were sequenced on an ABI 3730xl automatic sequencer. Sequence chromatograms were analyzed by Chromas software. Allele profiles were obtained by submitting sequences to the PubMLST *Clostridioides difficile* database; sequence types (STs) were assigned via the University College Cork MLST database. All isolates were subjected to MLST.

2.6 Antimicrobial-Susceptibility Testing

MICs of tetracycline, ciprofloxacin, cefoxitin, cefotaxime, vancomycin, clindamycin, erythromycin, levofloxacin, and metronidazole were determined by agar dilution following CLSI document M11-A8. *Clostridioides difficile* ATCC 700057 served as the quality-control strain. Interpretation followed CLSI M100 ED34. For antimicrobials without official CLSI clinical breakpoints, epidemiological cut-off values

(ECOFFs) were applied: ciprofloxacin ≥ 8 mg/L, erythromycin ≥ 8 mg/L, and levofloxacin ≥ 8 mg/L were defined as resistant. Vancomycin resistance was defined as > 2 mg/L according to EUCAST criteria.

2.7 Clinical-data Collection and Risk-factor Analysis

Collected variables: age, sex, fever, underlying diseases (hypertension, diabetes, hyperlipidemia, immunocompromised status), white-blood-cell count, serum albumin, serum creatinine, surgical history, ICU admission, length of hospital stay, self-care ability, disability duration, antibiotic exposure, chemotherapy, malignancy, cirrhosis. Patients were divided into colonization group and non-colonization group according to CD detection results. Variables with $P < 0.05$ in univariate analysis were entered into multivariate logistic regression to screen independent risk factors for CD colonization.

2.8 Statistical Analysis

Statistical analyses were performed using SPSS 22.0. Categorical variables were expressed as *n* (%), compared by χ^2 test. Continuous variables were expressed as mean ± standard deviation and compared by the *t*-test. Factors yielding $P < 0.05$ in univariate analysis were included in multivariate logistic regression. $P < 0.05$ was considered statistically significant.

3 Results

3.1 *C. difficile* Colonization Rate

C. difficile was isolated from 95 of 300 elderly hospitalized patients; the overall colonization rate was 31.67%.

3.2 Toxin-Gene Detection

Among the 95 C. *difficile* isolates, 41 (43.16%) were non-toxigenic (tcdA-/tcdB-) and 54 (56.84%) were toxigenic. All 54 toxigenic isolates were tcdA+/tcdB+, and no tcdA-/tcdB+ strains were detected.

3.3 MLST Results

All 95 C. *difficile* isolates were assigned to 15 distinct STs. ST81 was most prevalent (28 isolates, 29.47%), followed by ST39 (18 isolates, 18.95%). The remaining STs were ST3 (10, 10.53%), ST29 (8, 8.42%), ST42 (6, 6.32%), ST100 (6, 6.32%), ST15 (5, 5.26%), ST54 (5, 5.26%), ST2 (3, 3.16%), ST8 (1, 1.05%), ST12 (1, 1.05%), ST53 (1, 1.05%), ST254 (1, 1.05%), ST292 (1, 1.05%), and ST909 (1, 1.05%) (Table 1).

Table 1
Sequence type distribution of 95 C. difficile isolates

ST	No. of isolates (n)	Proportion (%)
ST81	28	29.47
ST39	18	18.95
ST3	10	10.53
ST29	8	8.42
ST42	6	6.32
ST100	6	6.32
ST15	5	5.26
ST54	5	5.26
ST2	3	3.16
ST8	1	1.05
ST12	1	1.05
ST53	1	1.05
ST254	1	1.05
ST292	1	1.05
ST909	1	1.05
Total	95	100.00

3.4 Antimicrobial Susceptibility

All 95 isolates were susceptible to vancomycin and metronidazole. Resistance rates were as follows: cefoxitin 96.84%, cefotaxime 88.42%, clindamycin 100.00%, ciprofloxacin 95.79%, levofloxacin 34.74%, tetracycline 56.84%, and erythromycin 31.58% (Table 2).

Table 2
Antimicrobial resistance of 95 colonizing CD isolates against nine agents

Antimicrobial	MIC min (mg/L)	MIC max (mg/L)	MIC50 (mg/L)	MIC90 (mg/L)	Resistant isolates(n)	Resistance rate (%)
Tetracycline	4	≥ 64	16	≥ 64	54	56.84
Ciprofloxacin	4	16	8	16	91	95.79
Cefoxitin	4	≥ 64	≥ 64	≥ 64	92	96.84
Cefotaxime	4	≥ 64	≥ 64	≥ 64	84	88.42
Vancomycin	0.25	1	0.5	0.5	0	0.00
Clindamycin	64	≥ 128	≥ 128	≥ 128	95	100.00
Erythromycin	≤ 1	≥ 128	2	> 128	30	31.58
Levofloxacin	≤ 2	≥ 64	4	≥ 64	33	34.74
Metronidazole	0.125	1	0.25	1	0	0.00

3.5 Risk-factor Analysis for CD Colonization in Elderly Hospitalized Patients

3.5.1 Univariate Analysis

No significant inter-group differences were observed for sex, fever, hypertension, diabetes, hyperlipidemia, white-blood-cell count, serum albumin, serum creatinine, surgical history, ICU admission, self-care-ability score, disability duration, chemotherapy, malignancy, or cirrhosis ($P > 0.05$). The colonization group showed significantly older age, higher proportion of immunocompromised subjects, longer hospital stay, and higher rate of antibiotic exposure compared with the non-colonization group ($P < 0.05$) (Table 3).

Table 3
Univariate analysis of risk factors for CD colonization among elderly hospitalized patients

Factor	Colonization (n = 95)	Non-colonization (n = 205)	t/χ^2 value	P value
Sex [n (%)]			0.179	0.673
Male	64 (67.37)	133 (64.88)		
Female	31 (32.63)	72 (35.12)		
Age (years, mean ± SD)	88.47 ± 4.98	85.19 ± 5.16	5.178	< 0.001
Fever [n (%)]	40 (42.11)	79 (38.54)	0.345	0.557
Hypertension [n(%)]	21 (22.11)	37 (18.05)	0.685	0.408
Diabetes [n (%)]	24 (25.26)	40 (19.51)	1.279	0.258
Hyperlipidemia [n (%)]	19 (21.05)	34 (16.59)	0.878	0.349
Immunocompromised [n(%)]	24 (25.26)	28 (13.66)	6.101	0.014
WBC (×10⁹/L, mean ± SD)	14.16 ± 3.64	13.58 ± 3.57	1.301	0.194
Serum albumin (g/L, mean ± SD)	34.45 ± 4.17	33.92 ± 4.06	1.043	0.298
Serum creatinine (μmol/L, mean ± SD)	89.13 ± 10.44	90.71 ± 10.62	1.205	0.229
Surgical history [n (%)]	18 (18.95)	33 (16.10)	0.374	0.541
ICU admission [n (%)]	29 (30.53)	53 (25.85)	0.714	0.398
Length of stay (days, mean ± SD)	41.78 ± 8.13	23.14 ± 7.62	19.293	< 0.001
Self-care ability (score, mean ± SD)	60.73 ± 10.11	62.45 ± 9.83	1.397	0.163
Disability duration (years, mean ± SD)	1.03 ± 0.36	0.97 ± 0.25	1.671	0.096
Antibiotic exposure [n (%)]	43 (45.26)	64 (31.22)	5.580	0.018
Chemotherapy use [n (%)]	9 (9.47)	16 (7.80)	0.237	0.627
Malignancy [n (%)]	11 (11.58)	19 (9.27)	0.385	0.535
Cirrhosis [n (%)]	6 (6.32)	8 (3.90)	0.850	0.357

3.5.2 Multivariate Logistic-Regression Analysis

Variables with $P < 0.05$ in univariate analysis (age, immunocompromised status, length of hospital stay, antibiotic exposure) were entered as independent variables; CD colonization served as dependent variable (colonization = 1, non-colonization = 0). Older age, immunocompromised status, prolonged hospital stay, and antibiotic exposure were identified as independent risk factors for CD colonization ($P < 0.05$) (Table 4, Table 5).

Table 4
Variable assignment for logistic-regression model

Dependent variable	Assignment	Independent variable	Assignment
CD colonization	colonization = 1, non-colonization = 0	Age	Continuous
		Immunocompromised	yes = 1, no = 0
		Length of stay	Continuous
		Antibiotic exposure	yes = 1, no = 0

Table 5
Multivariate logistic-regression for risk factors of CD colonization in elderly hospitalized patients

Variable	β	SE	Wald χ^2	OR	95% CI	P
Age	0.823	0.259	10.097	2.277	1.822–2.733	< 0.001
Immunocompromised	0.768	0.274	7.856	2.155	1.715–2.596	< 0.001
Length of stay	0.845	0.242	12.192	2.328	1.887–2.769	< 0.001
Antibiotic exposure	0.705	0.285	6.119	2.024	1.682–2.366	< 0.001
Constant	12.467	2.145	33.781	—	—	—

4 Discussion

This study reported a *C. difficile* colonization rate of 31.67% among 300 elderly hospitalized patients, higher than the 5%–19% asymptomatic carriage rate reported from French multicenter long-term-care-facility investigations [12], but within the range of 15%–50% reported among elderly long-term-care populations in prior studies [13, 14]. Notably, despite the absence of diarrheal symptoms upon admission, the high colonization rate indicates that elderly hospitalized patients constitute an important reservoir for asymptomatic *C. difficile* carriage, creating potential risk for nosocomial transmission and subsequent progression to CDI [15, 16].

Toxin-gene testing showed that 56.84% of isolates were toxigenic, and all toxigenic isolates were tcdA+/tcdB+. No tcdA-/tcdB+ strains were detected in this cohort. All toxigenic isolates carried both tcdA and tcdB. This finding indicates that in this cohort, toxin A detection alone would not have missed any toxigenic isolates[10]. However, given the regional heterogeneity of toxin gene profiles reported in China [4], including the predominance of the TcdA-negative ST81 clone in some regions [21], molecular assays detecting both tcdA and tcdB remain recommended for clinical laboratories.

MLST of all 95 C. difficile isolates yielded 15 STs; ST81 (29.47%) and ST39 (18.95%) predominated. ST81 has been increasingly reported as a prevalent sequence type in multiple hospitals in China [21]. A prospective study of four ICUs in a teaching hospital in Central South China reported ST3, ST39, and ST54 as the most prevalent sequence types (ST3: 34 isolates; ST39: 27 isolates; ST54: 19 isolates) [18]; epidemiological surveillance performed in Chongqing (2021–2022) similarly reported the prevalence of ST39/ST54 [19]. These findings suggest that ST39 represents an endemic C. difficile clone among elderly populations across multiple regions of China, while ST81 has emerged as a predominant clone in our cohort. By contrast, Current European surveillance data indicate that the predominant circulating ribotypes are RT014/020, RT002, and RT078[20]. Marked inter-regional divergence in ST distribution implies geographic variation in *C. difficile* population structures, possibly driven by differing antibiotic-utilization patterns, healthcare-system environments, and host-population characteristics [4, 21].

Susceptibility testing demonstrated universal susceptibility to vancomycin and metronidazole, consistent with international investigations [22, 23]. Therefore, empirical vancomycin- or metronidazole-based therapy for CDI remains reasonable before susceptibility results become available. However, severe resistance was observed for multiple drug classes: cefoxitin 96.84%, cefotaxime 88.42%, clindamycin 100.00%, and ciprofloxacin 95.79%. This finding merits clinical concern because cephalosporins and clindamycin are frequently prescribed for respiratory and anaerobic infections in elderly patients. High resistance likely reflects expansion of resistant clones under sustained antibiotic selective pressure [4, 21]. Erythromycin (31.58%) and levofloxacin (34.74%) retained moderate in vitro activity; tetracycline showed intermediate-level resistance (56.84%). Collectively, cephalosporins, clindamycin, and ciprofloxacin are not recommended for CDI treatment due to excessive resistance rates [22, 23].

It should be highlighted that our cohort consisted of asymptomatic carriers. The boundary between colonization and infection is not absolute: colonization is a prerequisite for CDI, yet not every colonized patient progresses to symptomatic disease. Colonizing and infecting isolates display both similarities and differences in molecular profiles.In our dataset, 56.84% of isolates were toxigenic, lower than the proportion reported from clinically overt CDI patients in Xi'an, China (95.5%) [24]. This discrepancy may reflect the asymptomatic nature of our cohort, in which non-toxigenic strains may be more frequently encountered. This observation supports the hypothesis that hypervirulent clones tend to produce symptomatic illness, whereas better-adapted genotypes such as ST81 and ST39 may establish persistent asymptomatic colonization among hospitalized elderly hosts [18, 19]. Susceptibility to vancomycin and metronidazole among colonizing isolates was consistent with clinical CDI isolates;

extremely high resistance against cephalosporins and clindamycin indicates that colonizing strains carry multidrug-resistance determinants and may become dominant infectious clones under antibiotic pressure [21]. Colonization and infection should be regarded as a continuum shaped by host immune competence, gut-microbiome homeostasis, and antibiotic exposure, rather than a simple binary classification of “non-pathogenic versus pathogenic.” Enhanced surveillance and preventive interventions should target asymptomatic carriers carrying risk factors (advanced age, immunocompromise, prolonged hospitalization, and antibiotic exposure) to hamper the transition from colonization to symptomatic CDI [15, 25].

Multivariate regression identified older age, immunocompromised status, longer hospital stay, and antibiotic exposure as independent risk factors for *C. difficile* colonization. Mean age in the colonization group (88.47 ± 4.98 years) was significantly higher than in the non-colonization group (85.19 ± 5.16 years); each unit increase in age increased colonization risk (OR = 2.277, 95% CI 1.822–2.733). The clinical prediction rule developed by Yang et al. for toxigenic C. difficile carriage in hospitalized older adults demonstrated a good negative predictive value (95.4%) in the validation cohort[26], consistent with our results. Immunocompromised elderly patients had approximately 2.2-fold higher colonization risk (OR = 2.155), confirming the importance of host immunity. Prolonged hospitalization represented another strong risk factor (OR = 2.328). A large matched case-control study within the US Veterans Affairs healthcare system by Silva et al. also documented prolonged hospitalization as an independent CDI risk factor [27]. Antibiotic exposure increased colonization risk roughly two-fold (OR = 2.024). The underlying mechanism may involve antibiotic-mediated disruption of commensal gut microbiota, loss of colonization resistance, and selection for overgrowth of resistant C. *difficile* strains [28].

These risk factors exert synergistic effects. Elderly patients frequently suffer multiple comorbidities, prolonged hospitalizations, and high antibiotic-exposure rates, further impairing immune function and creating a vicious cycle of “aging–hospitalization–antibiotic–immunosuppression,” amplifying C. *difficile* colonization and infection risk [25, 28]. Comprehensive prevention strategies should be implemented for high-risk populations: strict antibiotic-stewardship enforcement, avoidance of unnecessary prolonged hospitalization, immune-supportive care for elderly patients, and contact-precaution measures for asymptomatic carriers to interrupt nosocomial horizontal transmission [16, 20].

Limitations of this study: First, it was a single-center investigation with a limited sample size; generalizability requires validation through larger multicenter studies [4, 19]. Second, only cross-sectional colonization data were collected; no prospective follow-up was performed to observe progression from colonization to CDI. Third, MLST was not performed for non-toxigenic isolates; therefore, the differential roles of distinct STs in colonization versus infection cannot be fully evaluated. Fourth, operational definitions for immunocompromised status and the antibiotic-exposure time window were absent, reducing reproducibility. Fifth, binary-toxin genes were not tested.

5 Conclusion

Elderly hospitalized patients exhibit a high CD colonization rate, with toxigenic strains accounting for 56.84% of isolates; ST81 and ST39 constitute major local genotypes. Older age, immunocompromised status, prolonged hospital stay and antibiotic exposure are independent risk factors for CD colonization. Vancomycin and metronidazole remain preferred empirical therapeutic agents for CDI. Cephalosporins, clindamycin and ciprofloxacin demonstrate severe resistance and should be used cautiously among elderly patients to mitigate further resistance development and reduce CDI burden. Susceptibility data obtained from asymptomatic colonizing isolates should be extrapolated to clinical CDI with caution.

Declarations

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The authors declare no conflict of interest.

Data Availability

The datasets generated and/or analysed during the current study are available in the PubMLST Clostridioides difficile database, https://pubmlst.org/organisms/clostridioides-difficile (isolate IDs: 2430–2524).

Author Contributions

Hengchao Du and Xiaobo Xu designed the study. Chenfang Ma, Tao Lin, Chen Zhou and Qunfang Lei collected and analyzed the data. Qunyan Lei supervised the project and revised the manuscript. All authors contributed to writing and approved the final manuscript.

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Ethical Approval and Consent to Participate

This study was approved by the Ethics Committee of Zhejiang Rongjun Hospital (Approval Number: ZJRH-2021-034, Date: June 22, 2022)and was conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants prior to their inclusion in the study.

Conflict of Interest Declaration

The authors declare that they have no affiliations with or involvement in any organization or entity with any financial or non-financial interest in the subject matter or materials discussed in this manuscript.

Consent for Publication

Not applicable. This manuscript does not contain any identifiable patient images, personal clinical details, or any information that could compromise patient anonymity.

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