

# Establishment and Clinical Application of Rh Blood Group Bank in the East China Region

Yiming Jin<sup>1,\*</sup>, Xiaoyan Fu<sup>2,\*</sup>, Shuhong Xie<sup>3</sup>, Zhen Liu<sup>1</sup>, Zihao Xu<sup>1</sup>, Mingyuan Wang<sup>1</sup>, Yihan Wang<sup>1</sup>, Rong Lu<sup>1</sup>, Lingling Wang<sup>1</sup>

<sup>1</sup>Department of Blood Screening Test, Suzhou Blood Center, Suzhou, People's Republic of China; <sup>2</sup>Department of Blood Screening Test, Tai Cang Blood Branch Station, Tai Cang, People's Republic of China; <sup>3</sup>Division of Transfusion Medicine, Suzhou Blood Center, Suzhou, People's Republic of China

\*These authors contributed equally to this work

Correspondence: Lingling Wang; Rong Lu, Department of Blood Screening Test, Suzhou Blood Center, Suzhou, People's Republic of China, Email [linglingwang0716@163.com](mailto:linglingwang0716@163.com); [cv-25a@163.com](mailto:cv-25a@163.com)

**Purpose:** To investigate the expression and distribution of Rh phenotypes (C, c, D, E, e) among voluntary blood donors in a specific region of East China, to establish a regional Rh phenotype database, and to enhance the precision and efficacy of clinical blood transfusions.

**Patients and Methods:** A total of 28979 blood samples were collected from voluntary donors at a central blood station in East China between May 2023 and December 2023. An automated blood type analyzer was used to determine Rh phenotypes, which were then applied clinically for ABO and Rh blood type-matching in transfusions.

**Results:** Analysis of 28672 RhD-positive donors identified 13 RhD variants and eight Rh phenotypes, with the most common being CCee (42.69%) and CcEe (35.27%). Antigen frequencies were e (92.07%), C (87.85%), c (56.75%), and E (47.65%). Among 307 RhD-negative donors, seven Rh phenotypes were identified, with ccee (60.26%) and Ccee (29.32%) being the predominant ones. Antigen frequencies were e (99.67%), c (96.09%), C (34.53%), and E (6.84%). These findings supported 1834 ABO- and Rh- blood type-matching transfusions, but no significant difference was observed between ABO-compatible and dual-system compatible transfusions ( $p > 0.05$ ). Additionally, it was found that there are significant differences compared to populations from India and other regions ( $p > 0.05$ ).

**Conclusion:** In this region of East China, the prevalence of RhD variants among voluntary blood donors was 0.045%. The predominant Rh phenotypes were CCDee and CcDEe, with the highest frequencies observed for the e and C antigens. And the frequency of Rh phenotypes in this region differs from related studies in other areas. It is essential to strengthen the establishment of a rare blood type database in East China to provide data support for clinical compatible blood transfusion.

**Keywords:** Rh phenotype, Rh antigen, Rh antibody, compatible blood transfusion

## Introduction

The Rh blood group system is highly complex, ranking just behind the ABO blood group system in importance.<sup>1</sup> It includes 55 antigens, with C, c, D, E, and e being the most common and clinically significant. Antigenicity decreases in the order of  $D > E > C > c > e$ .<sup>2</sup> Clinically, blood types are classified as RhD-positive or RhD-negative based on the presence or absence of the D antigen. The “Clinical Transfusion Technical Specifications” require testing only for the ABO blood group and the D antigen in the Rh system prior to transfusion and do not mandate tests for the C, c, E, and e antigens.<sup>3</sup> The absence of Rh blood group compatibility testing can lead to the development of antibodies during transfusion, that the recipient’s immune system produces antibodies against foreign Rh antigens on transfused red blood cells (RBCs). In a Rh-negative individual receiving Rh-positive blood, the immune system recognizes Rh antigens as “non-self”, triggering an immune response and the production of anti-Rh antibodies, most commonly anti-D. This can result in hemolytic transfusion reactions (HTRs), characterized by RBC destruction, or in hemolytic disease of the fetus and newborn (HDFN) in pregnancy. These immune responses can cause significant clinical complications, including acute hemolysis, organ dysfunction, or fetal morbidity in Rh-negative individuals.<sup>4</sup> In addition to anti-D, antibodies



against other Rh antigens, such as anti-c and anti-E, although less common, can also result in transfusion reactions. These reactions are typically mediated by immunoglobulin G (IgG) antibodies, which lead to extravascular hemolysis. Recent reports have highlighted the potential for severe transfusion reactions, including acute hemolysis and organ dysfunction.<sup>5,6</sup> Therefore, Rh blood group typing in blood donors is crucial to reduce transfusion-related complications associated with the Rh system. Additionally, with advances in clinical electronic cross-matching technology, demand for comprehensive testing of antigens, antibodies, and genes in both blood products and patient blood group systems increases. Establishing a complete blood group gene database not only improves patient treatment and care for blood donors but also supports the recruitment of voluntary blood donors, emergency preparedness, and precise treatment of patients. Also, the development of genomic technologies (such as next-generation sequencing, NGS) can significantly enhance the database of blood group antigens by providing a more comprehensive and accurate understanding of genetic variations within the Rh system and other blood group antigens.<sup>7</sup> However, except for some provincial blood centers, most blood stations in China only conduct RhD antigen testing. Therefore, to further address the issue of blood shortage for individuals with rare blood groups and to improve the database of rare blood group, this study aims to investigate the frequency and distribution of Rh blood group antigens in the East China.

## Materials and Methods

### Blood Samples

Between May and December 2023, 28979 blood samples were collected via venipuncture from voluntary donors at a central blood station in East China. Blood group testing helped classify 9032 samples as type A, 7816 as type B, 9608 as type O, and 2523 as type AB, with ages of donors ranging from 18–55 years.

### Phenotype Detection

After centrifuging the EDTA-anticoagulated whole blood samples at  $1,200 \times g$  for 3 min, a 2–3% suspension of red blood cells was prepared. Rh blood group antigens (C, c, D, E, and e) were phenotyped using monoclonal antibody reagents (MoAbs; anti-C, 20223001; anti-c, 20223102; anti-E, 20223203; anti-e, 20223302; Shanghai Blood Biomedical Co, China. anti-D, 517234, Immucor, Norcross, GA, USA) on an automated blood typing analyzer (PK7300; Beckman Coulter, CA, USA). A positive reaction manifests as cells not settling naturally at the center of the gradient plate, whereas a negative reaction shows cells settled at the center. All procedures are strictly carried out in accordance with the blood station's standard operating procedures (SOPs).

### Rh-Negative Confirmation

For samples identified as RhD-negative, red blood cells were diluted to a 0.7–0.9% suspension in saline. Fifty microliters of this suspension were added to each well of the Rh blood group antigen test card microplate and centrifuged at 800 rpm ( $90 \times g$ ) for 2 min. A positive reaction is indicated by red blood cells present on or dispersed within the gel surface, and a negative reaction by cells settled at the bottom of the gel.

### Clinical Transfusion

In the clinical transfusion department, selected patients requiring multiple transfusions underwent Rh phenotype testing prior to receiving ABO- and Rh blood group-matched transfusions. Clinical data show that the majority of patients are diagnosed with Neoplasms of haematopoietic or lymphoid tissues. The efficacy of transfusions was evaluated by measuring hemoglobin levels 24–48 h post-transfusion, with an increase of approximately 10 g/L indicating effective transfusion. Hemoglobin is measured by colorimetric on hematology analyzer BC-6800 (Mindray, Shenzhen, China).

### Statistical Analysis

Statistical analysis was performed using the IBM SPSS Statistics software, version 19 (IBM Corp., Armonk, NY, USA). Differences were evaluated using the chi-squared test, with a p-value of less than 0.05 denoting statistical significance.

## Results

### Detection of RhD

A total of 28979 samples were analyzed, of which 28672 were RhD-positive, representing 98.94% of the donors. The remaining 307 samples were RhD-negative, representing 1.06% of all samples. Additionally, 13 samples exhibited RhD variants, representing 0.045% of the total samples analyzed.

### Clinical Efficacy of Red Blood Cell Transfusion

The clinical transfusion department selected 1834 patients requiring transfusion therapy to receive ABO- and Rh blood group-matched transfusions. The comparison of the efficacies of these transfusions is demonstrated in Table 1.

### Frequency Distribution of Rh Blood Group Antigens

Among the 28672 RhD-positive donors, the frequency distribution of the antigens (C, c, E, e) was  $e > C > c > E$  (Table 2). For the 307 RhD-negative donors, the descending order of antigen frequency was  $e > c > C > E$  (Table 3).

### Distribution of Rh System Phenotypes

In RhD-positive donors, eight distinct Rh phenotypes were identified with distribution frequencies descending as follows: CCee (42.68%), CcEe (35.27%), Ccee (9.18%), ccEE (7.78%), ccEe (3.89%), CCEe (0.57%), ccee (0.48%), and CcEE (0.15%). No CCEE phenotype was observed (Table 4).

**Table 1** Comparison of the Efficiency of Red Blood Cell Infusion

Age Groups	ABO System-Compatible Transfusion			ABO, Rh Blood Group Dual System-Compatible Transfusion		
	N	Effective Cases	Effective Rate (%)	N	Effective Cases	Effective Rate (%)
20–29	41	41	100	110	110	100
30–39	62	62	100	445	443	99.6
40–49	43	43	100	509	509	100
50–59	141	140	99.3	435	434	99.8
60–69	152	150	98.7	335	332	99.1
Total	439	436	99.3	1834	1828	99.7

**Notes:** The efficiency of red blood cell transfusion was compared between the two groups:  $\chi^2 = 1.19$ ,  $p > 0.05$ .

**Table 2** Frequency Distribution of RhD-Positive Blood Group Antigens (C, c, E, e)

Antigen	Group A (%)	Group B (%)	Group O (%)	Group AB (%)	Total (%)
C	7895 (27.54)	6745 (23.52)	8360 (29.16)	2188 (7.63)	25,188 (87.85)
c	5035 (17.56)	4433 (15.46)	5406 (18.85)	1397 (4.87)	16,271 (56.75)
E	4254 (14.84)	3690 (12.87)	4532 (15.81)	1186 (4.14)	13,662 (47.65)
e	8271 (28.85)	7092 (24.73)	8750 (30.52)	2285 (7.97)	26,398 (92.07)

**Table 3** Frequency Distribution of RhD-Negative Blood Group Antigens (C, c, E, e)

Antigen	Group A (%)	Group B (%)	Group O (%)	Group AB (%)	Total (%)
C	25 (8.14)	37 (12.05)	29 (9.45)	29 (9.45)	106 (34.53)
c	73 (23.78)	103 (33.55)	86 (28.01)	86 (28.01)	295 (96.09)
E	2 (0.65)	6 (1.95)	13 (4.23)	13 (4.23)	21 (6.84)
e	77 (25.08)	107 (34.85)	88 (28.66)	88 (28.66)	306 (99.67)

**Table 4** Phenotypic Distribution of RhD-Positive Systems

Phenotype	Group A (%)	Group B (%)	Group O (%)	Group AB (%)	Total (%)
CCee	3869 (13.49)	3232 (11.27)	4062 (14.17)	1076 (3.75)	12239 (42.68)
CcEe	3153 (11.00)	2721 (9.49)	3360 (11.72)	878 (3.06)	10112 (35.27)
Ccee	801 (2.79)	742 (2.59)	874 (3.05)	216 (0.75)	2633 (9.18)
ccEE	663 (2.31)	611 (2.13)	756 (2.64)	202 (0.70)	2232 (7.78)
ccEe	366 (1.28)	308 (1.07)	352 (1.23)	88 (0.31)	1114 (3.89)
CCEe	51 (0.18)	44 (0.15)	51 (0.18)	16 (0.06)	162 (0.57)
ccee	31 (0.11)	45 (0.15)	51 (0.18)	11 (0.04)	138 (0.48)
CcEE	21 (0.07)	6 (0.02)	13 (0.05)	2 (0.006)	42 (0.15)
Total	8955 (31.23)	7709 (26.87)	9519 (33.22)	2489 (8.68)	28672 (100)

**Table 5** Phenotypic Distribution of RhD-Negative Systems

Phenotype	Group A (%)	Group B (%)	Group O (%)	Group AB (%)	Total (%)
CCee	4 (1.30)	4 (1.30)	2 (0.65)	1 (0.33)	11 (3.58)
CcEe	0 (0)	4 (1.30)	0 (0)	0 (0)	4 (1.30)
Ccee	21 (6.84)	29 (9.45)	26 (8.47)	14 (4.56)	90 (29.32)
ccEE	0 (0)	0 (0)	1 (0.33)	0 (0)	1 (0.33)
ccEe	2 (0.65)	2 (0.65)	11 (3.58)	0 (0)	15 (4.89)
CCEe	0 (0)	0 (0)	1 (0.33)	0 (0)	1 (0.33)
ccee	50 (16.29)	68 (22.15)	48 (15.64)	19 (6.19)	185 (60.26)
Total	77 (25.08)	107 (34.85)	89 (28.99)	34 (11.07)	307 (100)

In RhD-negative donors, seven Rh phenotypes were identified, with distribution frequencies descending as follows: ccee (60.26%), Ccee (29.32%), ccEe (4.89%), CCee (3.58%), CcEe (1.30%), ccEE (0.33%), and CCEe (0.33%). Both CcEE and CCEE phenotypes were not observed (Table 5).

## Comparison of the Phenotype Distribution Frequencies with Other Regions and Ethnic Groups

The most common Rh phenotype in this study was CCDee (42.23%), Chi-square analysis showed a statistically significant difference compared to Han populations from mainland China<sup>8</sup> ( $\chi^2=106.03, p > 0.05$ ) and India<sup>9</sup> ( $\chi^2=11709.43, p > 0.05$ ). Due to the lack of complete and accurate data, statistical analysis with Caucasian and African<sup>10</sup> populations could not be performed. (Table 6).

**Table 6** Comparison of the Phenotype Frequencies of Population in East China With Other Regions and Ethnic Groups

Phenotype	This Study	Han population in Mainland China <sup>8</sup>	Caucasian <sup>10</sup>	Black <sup>10</sup>	India <sup>9</sup>
CCDee	42.23	40.72	18.5	3	40.95
CcDEe	34.89	38.46	13.3	4	14.537
CcDee	9.09	7.51	34.9	24	30.91
ccDEE	7.70	6.94	2.3	1	0.78
ccDEe	3.84	3.61	11.8	16	3.69
CCDEe	0.56	0.57	0.2	–	0.32
ccDee	0.48	0.35	2.1	42	1.15

(Continued)

**Table 6** (Continued).

Phenotype	This Study	Han population in Mainland China <sup>8</sup>	Caucasian <sup>10</sup>	Black <sup>10</sup>	India <sup>9</sup>
CcDEE	0.14	0.64	0.1	–	0.4
CCdee	0.04	0.14	–	–	0.05
CcdEe	0.01	0.42	0.05	–	0.075
Ccdee	0.31	0.21	0.8	1	2.32
ccdEE	0.00	–	–	–	0.004
ccdEe	0.05	–	0.8	–	0.05
CCdEe	0.00	–	–	–	–
ccdee	0.64	0.28	15.1	7	4.76

## Discussion

The International Society of Blood Transfusion (ISBT) has confirmed and named 45 human blood group systems, with ABO and Rh systems being the most relevant to transfusion practices.<sup>11</sup> Rh blood group antigens are encoded by two closely linked genes on chromosome 1: *RHD*, which encodes the RhD polypeptide, and *RHCE*, which encodes the RhCE polypeptide. These genes are tightly linked and arranged in opposite directions, exhibiting a high degree of homology.<sup>12</sup> Antibodies against the Rh blood group system account for over 50% of all blood group alloantibodies, with anti-E and anti-c being the most prevalent.<sup>13</sup> These antibodies predominantly belong to the IgG class and are closely associated with transfusion and pregnancy complications. In individuals with Rh-negative phenotypes who receive transfusions of Rh-positive red blood cells or are exposed to them, the body may be stimulated to produce the corresponding antibodies, which can persist for long periods.

The transfusion strategy currently employed in China is based on ABO and RhD typing, with compatible transfusion but without screening for C, c, E, or e antigens within the Rh system. Some patients develop antibodies against Rh factors following the transfusion of red blood cells with different Rh phenotypes, which increases the risk of alloimmunization and hemolytic transfusion reactions if they are subsequently transfused with blood containing the corresponding antigens. Domestic studies in China have reported that the incidence of irregular antibodies in patients who received Rh C, c, E, and e antigen-matched transfusions was 1.30%, with no adverse transfusion reactions observed. In contrast, the control group exhibited a 10.26% incidence of irregular antibodies, primarily delayed serological reactions, showing a significant difference between the two groups.<sup>14</sup> A retrospective analysis of Rh antigen compatibility between blood donors and recipients indicated that the probability of Rh antigen-incompatible transfusions is highest when recipients receive blood from multiple donors.<sup>15</sup> Consequently, for patients requiring repeated transfusions, Rh-matched transfusion is essential to prevent and reduce alloimmune reactions caused by the Rh system. Table 1 illustrates the successful implementation of ABO and Rh dual-system matched transfusions in 1834 patients who required multiple transfusions, achieving an efficacy rate of 99.7% for red blood cell transfusions. Of these, 439 had previously received routine ABO-compatible transfusions, with a red blood cell transfusion efficacy rate of 99.3%. Statistical comparisons between the two groups revealed no significant differences in transfusion efficacy ( $p > 0.05$ ). However, the clinical case coverage in this study was insufficient to include all patients who received transfusions. Given the variability in patient conditions and individual differences, more rigorous controlled trials are necessary to gain further insight, this will be the focus of our next study.

We observed that the proportion of RhD-negative blood donors was 1.06%, which is significantly higher than the 0.3–0.4% observed in the RhD-negative Han Chinese population. Table 2 and Table 3 indicate that among RhD-positive blood donors, the highest positivity rate was for the e antigen (92.07%), followed by the C antigen (87.85%) and the c antigen (56.75%), with the E antigen having the lowest positivity rate (47.65%). In RhD-negative donors, the highest positivity rate was for the e antigen (99.67%), followed by the c antigen (96.09%) and the C antigen (34.53%), with the E antigen again showing the lowest positivity rate (6.84%). The distribution of blood types A, B, and O among the study participants was relatively even, whereas the AB type was less common,

aligning with the ABO blood type distribution in the general population. Moreover, the frequency distribution of Rh antigens within each blood type was consistent with the overall frequency pattern, namely  $e > C > c > E$ , in RhD-positive blood. The overall negativity rate for the E antigen was 52.35% in RhD-positive blood and 93.16% in RhD-negative blood. This finding is consistent with the highest proportion of anti-E antibodies among Rh blood group irregularities reported in the literature.<sup>16,17</sup> Furthermore, our findings revealed that the positivity rates for the C and e antigens were substantially elevated in RhD-positive individuals compared with that in RhD-negative individuals. This suggests that RhD-positive individuals are more likely to develop anti-c and anti-E antibodies following transfusion. Individuals who are RhE-negative are particularly susceptible to producing anti-E antibodies when exposed to RhE-positive red blood cells, either through transfusion or during pregnancy, increasing the risk of transfusion-related complications. Several reports have documented hemolytic diseases resulting from these conditions.<sup>18–20</sup>

The results presented in Table 4 and Table 5 reveal eight distinct Rh phenotypes among the RhD-positive samples: CCee, CcEe, Ccee, ccEE, ccEe, CCEe, ccee, and CcEE. The most prevalent phenotypes were CCee and CcEe, found in 42.68% and 35.27% of cases, respectively. The other phenotypes were found at the following frequencies: Ccee (9.18%), ccEE (7.78%), ccEe (3.89%), CCEe (0.57%), ccee (0.48%), and CcEE (0.15%). The distribution of Rh phenotypes among the four blood types (A, B, O, and AB) exhibited a similar pattern, consistent with findings reported in related literature from various regions.<sup>21,22</sup> These data indicate minimal regional variation in RhD-positive phenotypes among the Han Chinese population, with CCee and CcEe being the most prevalent. In contrast, among RhD-negative blood donors, the antigen distribution frequency followed the order of  $e > c > C > E$ , with ccee and Ccee being the predominant phenotypes. The distribution of phenotypes differed between RhD-negative and RhD-positive donors. In our study, only seven phenotypes were identified among the RhD-negative donors, with no observed CCEE phenotypes. This indicates that the CCEE phenotype is rare among RhD-negative donors in this region; The absence of certain rare phenotypes, such as CCEE and CcEE, in this study is notable. In mainland China, the prevalence of CCEE is extremely low, with reports indicating it constitutes only 0.14% of the population.<sup>8</sup> Additionally, among over 2,000 Rhd cases in Xi'an, no CcEE phenotype has been identified, further emphasizing its rarity.<sup>23</sup> The low frequency of such phenotypes in the population presents significant challenges in transfusion medicine. Patients with these rare blood types often struggle to find compatible blood, which increases the risk of transfusion-related complications. Furthermore, the results presented in Table 6 reveal significant differences when compared to the Han population in other regions of mainland China, as well as populations in India and other areas. This suggests that the Rh phenotype frequency distribution in East China exhibits distinct regional characteristics. This underscores the importance of continued research into the genetic distribution of blood group antigens and the need for enhanced blood donor matching systems to mitigate the potential risks for individuals with rare blood phenotypes.

## Conclusion

Our study on Rh blood group expression and distribution characteristics among voluntary blood donors in the region has led to the establishment of a database of Rh phenotypes. This database provides crucial information to support local blood screening strategies and clinical blood safety. Currently, we have provided blood products with specific Rh typing for clinical use. Moving forward, it is essential to conduct testing for multiple blood group systems in the diverse ethnic populations of East China to further ensure the safety of clinical blood transfusions and minimize the wastage of blood resources.

## Data Sharing Statement

Data will be made available on request.

## Ethics Approval and Informed Consent

This study complies with the Declaration of Helsinki and was reviewed and approved by the Medical Ethics Committee of the Suzhou Blood Center (approval number: 202202, dated May 6, 2022). Written informed consent was obtained from all participants prior to their inclusion in the study.

## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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## Disclosure

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

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