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Research article

Distribution of virulence genes and antimicrobial resistance of *Escherichia coli* isolated from hospitalized neonates: A multi-center study across China

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A R T I C L E I N F O

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A B S T R A C T

Background: Escherichia coli is the most common gram-negative pathogen to cause neonatal infections. Contemporary virulence characterization and antimicrobial resistance (AMR) data of neonatal *E. coli* isolates in China are limited.

Methods: A total of 159 *E. coli* strains isolated from neonates were collected and classified into invasive and non-invasive infection groups, according to their site of origin. The presence of virulence genes was determined using polymerase chain reaction (PCR). All the strains were subjected to antimicrobial susceptibility testing using the broth dilution method.

Results: The top three virulence genes with the highest detection rates were *fimH* (90.6 %), *iutA* (88.7 %), and *kspMT* II (88.1 %). The prevalences of *fyuA* ($p = 0.023$), *kpsMT* K1 ($p = 0.019$), *ibeA* ($p < 0.001$), and *iroN* ($p = 0.027$) were significantly higher in the invasive infection group than in the non-invasive infection group. Resistance to ceftaxizime, sulfamethoxazole/trimethoprim, and ciprofloxacin was 75.5 %, 65.4 %, and 48.4 %, respectively. Lower rates of resistance to ceftazidime ($p = 0.022$), cefepime ($p = 0.005$), ticarcillin/clavulanic acid ($p = 0.020$) and aztreonam ($p = 0.001$) were observed in the invasive infection group compared to the non-invasive infection group. The number of virulence genes carried by *E. coli* was positively correlated with the number

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of antibiotics to which the isolates were resistant ($r = 0.71$, $p = 0.016$), and a specific virulence gene was associated with resistance to various species of antibiotics.
Conclusions: Neonatal *E. coli* isolates carried multiple virulence genes and were highly resistant to antibiotics. Further studies are needed to understand the molecular mechanisms underlying the association between virulence and AMR.

1. Introduction

Escherichia coli is a multifaceted microbe since some are commensals, normally inhabiting the intestinal tracts of both humans and animals whereas others are pathogenic responsible for a wide range of intestinal and extra-intestinal infections. Pathogenic *E. coli* can be broadly divided into two groups: intestinal pathogenic *E. coli* (InPEC) and extra-intestinal pathogenic *E. coli* (ExPEC) [1]. *E. coli* is the most common gram-negative pathogen to cause neonatal infections worldwide and has been implicated as the main causative agent of severe infectious diseases in newborns (including sepsis and bacterial meningitis), especially in developing countries [2,3].

E. coli can cause infections and diseases through various mechanisms including adhesion and colonization, toxin production, cellular invasion, and immune escape [4]. Several specific virulence factors (VFs), such as adhesins, invasins, iron-acquisition systems, protectines/serum resistance and toxins, have been identified in ExPEC strains, which evade host defenses, invade host tissues and ultimately elicit host inflammation [5,6]. VFs are either encoded by virulence genes on the bacterial chromosome, where they are usually located within pathogenicity islands (PAIs), or on plasmids [7]. PAIs are a group of large (>10 kb) integrative elements that accumulate virulence genes in specific regions of the bacterial chromosome, which can be propagated in bacterial populations via horizontal transfer to promote bacterial evolution [8].

The burden of antimicrobial resistance (AMR) in *E. coli* is severe, particularly with the emergence of multidrug-resistant (MDR) isolates, which have become one of the major public health threats in the twenty-first century [9,10]. AMR and virulence are not two independent characteristics, but rather a negative or positive relationship exists between them [11]. This relationship provides advantages to microorganisms, endowing them with characteristics that enable them to survive in different niches with different selective pressures (such as the presence of antibiotics) [12]. In the evolution of pathogens, the correlation between AMR and virulence has become an enormous concern, which could cause successful highly virulent and resistant clones such as ST131 being disseminated worldwide [12,13].

There is a paucity of large-scale, neonatal-specific studies on the distribution of virulence and AMR in *E. coli*. Therefore, knowledge regarding these data is essential for the optimal management of neonatal infections. In this study, we evaluated virulence genes distribution of *E. coli* in hospitalized neonates across China and determined their correlation with AMR.

2. Materials and methods

2.1. Collection of clinical strains

Clinical samples were collected from neonates (under 28 days of age) at six tertiary hospitals in six cities across China. A total of 159 strains were obtained, of which 60, 53, 18, 15, nine, and four were isolated from Zhengzhou, Kunming, Beijing, Suzhou, Shenzhen, and Hohhot, respectively. Patients hospitalized between November 2019 and October 2020, with positive *E. coli* cultures from any of the specimens, pharyngeal swabs, sputum, blood, cerebrospinal fluid, urine, ascites, or peripherally inserted central catheter (PICC) tips were included in this study. Specimens were collected from patients who matched these conditions: blood was collected from sepsis, cerebrospinal fluid was collected from meningitis, pharyngeal swabs and sputum was collected from lower respiratory tract infection, ascites was collected from intraperitoneal infection, and the PICC tips were collected from catheter-related blood infection. The specimens were stored in a freezer at −80 °C. The study was conducted in accordance with the Declaration of Helsinki, met all ethical requirements, and was approved by the Ethics Committee of Beijing Children's Hospital.

2.2. Strain identification and antimicrobial susceptibility testing

Clinical specimens were inoculated into MacConkey agar plate medium (CM00078) using the three-zone delineation method and incubated at 37 °C for 24h. The colonies were inoculated into *E. coli* chromogenic medium (EC166, Beijing Landbridge Technology Co., Ltd.) and cultured at 37 °C for 24h, and the blue colony phenotype indicated the presence of *E. coli* isolate. Isolated and purified strains were preserved in the freezer, at −80 °C [14].

Antimicrobial susceptibility testing was performed using the broth microdilution method, according to the instructions of the Sensititre™ Gram-Negative GNX2F Plate (Thermo Fisher Scientific, USA). Briefly, 3–5 colonies were mixed with H_2O to reach a McFarland value of 0.5 using a nephelometer, and 10 μL of the suspension was mixed into Sensititre Mueller Hinton Broth. The plate was inoculated with 50 μL of the suspension per well, using an 8-channel pipette. The Sensititre plate was sealed and incubated at 34–36 °C in a Sensititre ARIS 2X† for 18–24 h. In total, 21 antimicrobial agents were evaluated, namely: ticarcillin/clavulanic acid, piperacillin/tazobactam, ceftazidime, cefotaxime, cefepime, aztreonam, ertapenem, doripenem, meropenem, imipenem, tobramycin, gentamicin, amikacin, levofloxacin, ciprofloxacin, doxycycline, tigecycline, minocycline, sulfamethoxazole/trimethoprim, polymyxin B, and colistin. *E. coli* ATCC 25922 was used as the quality control strain. Antimicrobial susceptibility testing results were classified as

susceptible (S), intermediate (I), or resistant (R), in accordance with Clinical and Laboratory Standards Institute (CLSI) 2023 standards [15].

According to the international classification of the different degrees of multi-resistance [16], *E. coli* isolates were classified as follows: multidrug-resistant (MDR) was defined as acquired non-susceptibility to at least one agent in more than three antimicrobial categories, and extensively drug-resistant (XDR) was defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories. Pandrug resistance (PDR) was defined as non-susceptibility to all agents in all antimicrobial categories.

2.3. Detection of virulence genes

DNA was extracted using a bacterial genomic DNA extraction kit (Tiangen Biotech Co., Ltd., Beijing, China). Virulence genes were detected using polymerase chain reaction (PCR), including genes associated with adhesins (*papA*, *papG*, *papEF*, *sfa/focDE*, *papC*, *afa/draBC*, *fimH*), iron acquisition (*fyuA*, *iutA*, *iroN*), capsule synthesis/immune evasion (*kpsMT* K1, *kpsMT* II, *kpsMT* III, *traT*, *rfc*), toxins (*hlyA*, *cnf1*), invasion (*ibeA*), and pathogenicity-associated island (PAI-I_{CFT073}) [17,18]. The PCR was performed in a 25 μL mixture comprised of 12.5 μL of 2 × Taq PCR Mix, 1 μL of upstream and downstream primers, 1 μL of DNA template and 9.5 μL of sterilized ultrapure water. The program for PCR amplification was as follows: pre-denaturation at 93 °C for 5 min, denaturation at 93 °C for 30 s, annealing at a temperature determined individually for each gene (Table 1), and extension at 65 °C for 30 s. Thirty cycles were performed, followed by incubation at 72 °C for 5 min. The PCR products were identified by agarose gel electrophoresis and compared to a 100-bp ladder to confirm their appropriate size. The presence of a band of the expected size was considered as positivity to the

Table 1
Specific primers used in this study.

Target	Primer direction	Sequence (5' → 3')	Annealing temperature (°C)	Product length (bp)
PAI-I_{CFT073}[f]	Forward	GGACATCCTGTTACAGCGCGCA	65	930 [18]
	Reverse	TCGCCACCAATCACAGCGAAC		
papA[a]	Forward	ATGGCAGTGGTGTCTTTTGGTG	60	720 [17]
	Reverse	CGTCCCACCATACGTGCTCTTC		
papG allele II[a]	Forward	GGGATGAGCGGGCCTTTGAT	64	190 [17]
	Reverse	CGGGCCCCCAAGTAACTCG		
papEF[a]	Forward	GCAACAGCAACGCTGGTTGCATCAT	60	336 [17]
	Reverse	AGAGAGAGCCACTCTTATACGGACA		
fyuA[b]	Forward	TGATTAACCCCGCGACGGGAA	60	880 [17]
	Reverse	CGCAGTAGGCACGATGTTGTA		
kpsMT K1[c]	Forward	TAGCAAACGTTCTATTGGTGC	56	153 [18]
	Reverse	CATCCAGACGATAAGCATGAGCA		
hlyA[d]	Forward	AACAAGGATAAGCACTGTTCTGGCT	62	1177 [17]
	Reverse	ACCATATAAGCGGTCATTCCCGTCA		
sfa/focDE[a]	Forward	CTCCGGAGAACTGGGTGCATFTTAC	63	410 [17]
	Reverse	CGGAGGAGTAATTACAAACCTGGCA		
kpsMT II[c]	Forward	GCGCATTTGCTGATACTGTTG	60	272 [17]
	Reverse	CATCCAGACGATAAGCATGAGCA		
kpsMT III[c]	Forward	TCCTCTTGCTACTATTCCCCCT	60	392 [17]
	Reverse	AGGCGTATCCATCCCTCCTAAC		
iutA[b]	Forward	GGCTGGACATCATGGGAACTGG	64	300 [17]
	Reverse	CGTCGGGAACGGGTAGAATCG		
papC[a]	Forward	GTGGCAGTATGAGTAATGACCGTTA	60	200 [17]
	Reverse	ATATCCTTTCTGCAGGGATGCAATA		
fimH[a]	Forward	CACTCAGGGAACCATTCAGGCA	58	975 [18]
	Reverse	CTTATTGATAAACAAAAGTCAC		
afa/draBC[a]	Forward	GGCAGAGGGCCGGCAACAGGC	60	559 [17]
	Reverse	CCCGTAACGCGCCAGCATCTC		
traT[c]	Forward	GGTGTGGTGCGATGAGCACAG	64	290 [17]
	Reverse	CACGGTTCAGCCATCCCTGAG		
cnf1[d]	Forward	AAGATGGAGTTTCCTATGCAGGAG	64	498 [17]
	Reverse	CATTCAGAGTCCTGCCCTCATTATT		
rfc[c]	Forward	ATCCATCAGGAGGGGACTGGA	58	788 [17]
	Reverse	AACCATACCAACCAATGCGAG		
ibeA[e]	Forward	AGGCAGGTGTGCGCCGCGTAC	60	170 [17]
	Reverse	TGGTGCTCCGGCAAACCATGC		
iroN[b]	Forward	AAGTCAAAGCAGGGGTTGCCCG	60	667 [18]
	Reverse	GACGCCGACATTAAGACGCAG		

Note.
[a] adhesins-related.
[b] iron acquisition-related.
[c] capsule synthesis/immune evasion-related.
[d] toxins-related.
[e] invasin-related.
[f] pathogenicity-associated island.

Table 2
Prevalence of virulence genes in neonatal *E. coli* isolates, and virulence gene frequencies among invasive infection group compared to those with non-invasive infection group.

Virulence genes	Invasive infection group n = 48 (%)	Non-invasive infection group n = 111 (%)	Total n = 159 (%)	*p*
PAI-I_{CFT073}	40 (83.3)	81 (73.0)	121 (76.1)	0.224
papA	27 (56.3)	66 (59.5)	93 (58.5)	0.729
papG allele II	32 (66.7)	80 (72.1)	112 (70.4)	0.571
papEF	23 (47.9)	64 (54.7)	87 (54.7)	0.299
fyuA	46 (95.8)	91 (82.0)	137 (86.2)	**0.023**
kpsMT K1	23 (50.0)	34 (30.1)	57 (35.8)	**0.019**
hlyA	17 (35.4)	43 (38.7)	60 (37.7)	0.725
sfa/focDE	22 (45.8)	50 (45.0)	72 (45.3)	1.000
kpsMT II	44 (91.7)	96 (86.5)	140 (88.1)	0.434
kpsMT III	3 (6.2)	5 (4.5)	7 (4.4)	0.699
iutA	44 (91.7)	97 (87.4)	141 (88.7)	0.588
papC	27 (56.3)	54 (48.6)	81 (50.9)	0.394
fimH	46 (95.8)	98 (88.3)	144 (90.6)	0.235
afa/draBC	5 (10.4)	35 (31.5)	40 (25.2)	**0.005**
traT	37 (77.1)	88 (79.3)	125 (78.6)	0.834
cnf1	20 (41.7)	39 (35.1)	59 (37.1)	0.477
ibeA	13 (27.1)	3 (2.7)	16 (10.1)	**0.000**
rfc	25 (52.1)	60 (54.1)	85 (53.5)	0.864
iroN	42 (87.5)	79 (71.2)	121 (76.1)	**0.027**

Note: bold values indicate virulence genes with significant differences among the two groups ($p < 0.05$).

presence of the corresponding gene. *E. coli* ATCC 25922 was used as the standard quality control strain.

2.4. Definitions and strain grouping

The strains were categorized into invasive and non-invasive infection groups based on the site of isolation and whether the specimen was sterile body fluid or not [19–21]. Strains isolated from sterile body fluids such as blood, cerebrospinal fluid, and ascites were categorized into the invasive infection group, and strains isolated from sputum, pharyngeal swabs, and urine were categorized into the non-invasive infection group.

2.5. Statistical analysis

SPSS 23.0 software (IBM SPSS, Chicago, IL, USA) was used for data processing and analysis. Count data were expressed as proportions (%), and Fisher' s exact test was used for comparisons between groups. Pearson's correlation (*r*) coefficient and a binary logistic regression model were used to analyze the correlation between virulence genes and AMR. Differences with $p < 0.05$ were considered to be statistically significant.

Table 3
Susceptibility of 159 *E. coli* strains to 21 antibiotic agents (%).

Antibiotic agent		R (%)	I (%)	S (%)
Carbapenems	Imipenem	7 (4.4)	1 (0.6)	151 (95.0)
	Ertapenem	15 (9.4)	1 (0.6)	143 (90.0)
	Doripenem	7 (4.4)	2 (1.2)	150 (94.3)
	Meropenem	10 (6.3)	2 (1.2)	147 (92.5)
Sulfonamides	Sulfamethoxazole/trimethoprim	104 (65.4)	0 (0)	55 (34.6)
Cephalosporins	Ceftazidime	35 (22.0)	20 (12.6)	104 (65.4)
	Cefotaxime	120 (75.5)	2 (1.2)	37 (23.2)
	Cefepime	40 (25.2)	0 (0)	119 (74.8)
Aminoglycosides	Tobramycin	19 (11.9)	31 (19.5)	109 (68.6)
	Gentamicin	47 (29.6)	2 (1.2)	110 (69.2)
	Amikacin	0 (0)	0 (0)	159 (100.0)
Penicillins	Ticarcillin/clavulanic acid	26 (16.4)	54 (34.0)	79 (49.7)
	Piperacillin/tazobactam	16 (10.1)	4 (2.5)	139 (87.4)
Quinolones	Levofloxacin	73 (45.9)	86 (54.1)	0 (0)
	Ciprofloxacin	77 (48.4)	13 (8.2)	69 (43.4)
Monobactams	Aztreonam	62 (39.0)	17 (10.7)	80 (50.3)
Tetracyclines	Doxycycline	72 (45.3)	42 (26.4)	45 (28.3)
	Tigecycline	0 (0)	0 (0)	159 (100.0)
	Minocycline	20 (12.6)	23 (14.5)	116 (72.6)
Polypeptides	Colistin	1 (0.6)	0 (0)	158 (99.4)
	Polymyxin B	0 (0)	91 (57.2)	68 (42.8)

Table 4
AMR in invasive and non-invasive infection groups.

Antibiotic	Invasive infection group (n = 48)	Non-invasive infection group (n = 111)	Total (n = 159)	*p*
Sulfamethoxazole/trimethoprim	34 (70.8)	70 (63.1)	104 (65.4)	0.370
Ceftazidime	5 (10.4)	31 (27.9)	36 (22.6)	**0.022**
Cefotaxime	31 (64.6)	88 (79.3)	119 (74.8)	**0.072**
Cefepime	5 (10.4)	35 (31.5)	40 (25.2)	**0.005**
Gentamicin	11 (22.9)	36 (32.4)	47 (29.6)	0.260
Ticarcillin/clavulanic acid	3 (6.3)	24 (21.6)	27 (17.0)	**0.020**
Levofloxacin	21 (43.8)	53 (47.7)	74 (46.5)	0.730
Ciprofloxacin	22 (45.8)	55 (49.5)	77 (48.4)	0.731
Aztreonam	10 (20.8)	53 (47.7)	63 (39.6)	**0.001**
Doxycycline	23 (47.9)	49 (44.1)	72 (45.3)	0.730

Note: bold values indicate antibiotics with significant differences among the two groups ($p < 0.05$).

3. Results

3.1. General characteristics of E. coli strains

We identified 159 *E. coli* strains isolated from neonates that provided microbiologic data from 2019 through 2020. Among the 159 strains, 34 were from blood, 11 from cerebrospinal fluid, 2 from ascites, 1 from the PICC tip, 86 from sputum, 16 from pharyngeal swabs, and 9 from urine. The strains were categorized into two groups according to their isolation sites, the invasive infection group (n = 48) from blood, cerebrospinal fluid, ascites, and the PICC tip, and the non-invasive infection group (n = 111) from sputum, pharyngeal swabs, and urine.

3.2. Prevalence of virulence genes

In this study, 159 strains of *E. coli* were tested for the presence of genes encoding 19 VFs. The most frequently detected *E. coli* virulence gene was *fimH* (90.6 %, 144/159), followed by *iutA* (88.7 %, 141/159), *kspMT* II (88.1 %, 140/159), and *fyuA* (86.2 %, 137/159). Only *kpsMT* III had a distribution of less than 10 % (4.4 %, 7/159). The virulence genes with higher detection rates in the invasive infection group were *fyuA* (95.8 %, 46/48), *fimH* (95.8 %, 46/48), and *iutA* (91.7 %, 44/48), and the corresponding genes in the non-invasive infection group were *fimH* (88.3 %, 98/111), *iutA* (87.4 %, 97/111), and *kspMT* II (86.5 %. 96/111). A comparison of the invasive and non-invasive infection groups revealed that the prevalence of *fyuA* ($p = 0.023$), *kpsMT* K1 ($p = 0.019$), *ibeA* ($p < 0.001$), and *iroN* ($p = 0.027$) were significantly higher in the invasive infection group than in the non-invasive infection group, whereas that of *afa/draBC* ($p = 0.005$) was significantly lower in the invasive infection group than in the non-invasive infection group (Table 2).

Table 5
Logistic regression analysis of the relationship between virulence genes and AMR.

Antibiotic	Virulence gene	Regression coefficient (β)	*p*	Odds ratio (OR)	OR 95 % confidence interval (CI)
Sulfamethoxazole/trimethoprim	*papA*	−0.897	0.033	0.408	0.179–0.931
	hlyA	−0.894	0.022	0.409	0.190–0.931
Ceftazidime	*afa/draBC*	0.983	0.019	2.672	1.175–6.078
	iroN	−1.000	0.019	0.368	0.160–0.847
Cefotaxime	*kpsMT* K1	−0.875	0.029	0.417	0.190–0.914
	papC	1.017	0.018	2.766	1.191–6.424
	iroN	−1.248	0.037	0.287	0.089–0.928
	rfc	1.189	0.005	3.284	1.431–7.537
Gentamicin	*sfa/focDE*	−1.365	0.001	0.255	0.118–0.552
Ticarcillin/clavulanic acid	*papA*	−1.553	0.002	0.212	0.081–0.556
	sfa/focDE	1.082	0.035	2.951	1.081–8.050
	iroN	−1.060	0.037	0.346	0.128–0.937
Levofloxacin	*papA*	−1.645	0.000	0.193	0.092–0.407
	kspMT III	1.674	0.010	5.335	1.497–19.010
	fimH	1.679	0.028	5.361	1.197–24.009
	traT	−1.285	0.005	0.277	0.114–0.673
Ciprofloxacin	*papA*	−1.611	0.000	0.200	0.089–0.447
	kspMT III	2.064	0.050	7.875	0.991–62.555
	fimH	1.935	0.014	6.927	1.472–32.604
	traT	−1.572	0.002	0.208	0.076–0.563
	cnf1	−1.153	0.006	0.316	0.138–0.719
	rfc	1.156	0.006	3.176	1.397–7.220
Doxycycline	*sfa/focDE*	0.983	0.010	2.673	1.262–5.663
	hlyA	−0.966	0.015	0.380	0.175–0.828
	rfc	−0.747	0.040	0.474	0.232–0.967

3.3. AMR

Of the 21 antibiotics tested, the neonatal *E. coli* isolates exhibited a high rate of resistance to cefotaxime (75.5 %, 120/159), trimethoprim/sulfamethoxazole (65.4 %, 104/159) and ciprofloxacin (48.4 %, 77/159). No isolates were resistant to amikacin, tigecycline, polymyxin B, and only one was resistant to colistin, and all were more than 90 % susceptible to carbapenems (Table 3). Applying the classification of multiple degrees of resistance, we observed that 61.6 % (98/159) of the isolates were MDR, 10.1 % (16/159) were XDR, and none were PDR. Regarding the distribution of resistance in the invasive and non-invasive infection groups, we found that the invasive infection group had lower rates of AMR to ceftazidime ($p = 0.022$), cefepime ($p = 0.005$), ticarcillin/clavulanic acid ($p = 0.020$), and aztreonam ($p = 0.001$) (Table 4).

3.4. Correlation between virulence genes and AMR

We found a positive correlation between the total number of virulence genes and the number of antibiotics to which the isolates were resistant ($r = 0.71$, $p = 0.016$). Table 5 summarizes the correlation between distinct virulence genes and AMR in *E. coli* strains isolated from neonates. AMR affected by virulence includes resistance to sulfamethoxazole/trimethoprim, ceftazidime, cefotaxime, gentamicin, ticarcillin/clavulanic acid, levofloxacin, ciprofloxacin, and doxycycline. The strongest positive correlation was observed between *kspMT* III and ciprofloxacin (OR, 7.875; 95 % CI, 0.991 to 62.555), and the strongest negative correlation was observed between *papA* and levofloxacin (OR, 0.193; 95 % CI, 0.092 to 0.407). Resistance to quinolones was elevated in strains harboring *kspMT* III and *fimH* and decreased in strains harboring *papA* and *traT*. Strains with detectable *rfc* had higher resistance to cefotaxime and ciprofloxacin and lower resistance to doxycycline. Furthermore, *sfa/focDE* positively correlated with ticarcillin/clavulanic acid and doxycycline resistance and negatively correlated with gentamicin resistance. For strains with detectable *cnf1* and *kpsMT* K1, resistance to ciprofloxacin and cefotaxime was reduced, respectively.

4. Discussion

Neonates, especially preterm infants, face a higher risk of infection because of the immaturity and immunologic incompetence of the immune system [22], and *E. coli* is the main cause of serious infections in term and preterm infants [23]. In this study, we retrospectively investigated the prevalence of virulence genes and AMR in *E. coli* isolates from hospitalized neonates in a multicenter setting across China.

Data are very limited on the prevalence of virulence genes in neonatal *E. coli* strains. Our study showed that neonatal *E. coli* isolates carried multiple virulence genes, the most common of which were *fimH*, *iutA*, *kspMT* II, and *fyuA*, consistent with the findings of Cole et al. [24]. When grouped based on specimen source, *fyuA*, *fimH*, and *iutA* had higher positive detection rates in the invasive infection group, whereas *fimH*, *iutA*, *kspMT* II had higher positive detection rates in the non-invasive infection group.

FimH is a type 1 fimbrial adhesin with strong affinity to mannosylated glycoproteins of the urinary tract epithelium and CD48 receptor of human brain microvascular endothelial cells (HBMECs), enabling bacteria invasion, colonization, proliferation and subsequent formation of biofilm-like intracellular bacterial communities (IBCs) within host cells, which act as an important VF for adherence and invasion of extra-intestinal tissues [5,25]. In this study, the *fimH* detection rate was high in both groups, which suggests possible horizontal transfer from different sources or tissues as well as adhesion and invasion of cells in the respiratory tract, urinary tract, blood, cerebrospinal fluid, or other tissues [26]. *IutA* and *fyuA*, which are involved in iron metabolism, were also prevalent. In addition, *kpsMT* II encodes group 2 capsules (e.g., K1, K2, K5, and K15), which act as the protection factor against phagocytosis and the spreading factor [27]. Group 2 capsules predominate in ExPEC isolates and are considered to be required to prevent the host immune system [28].

Comparing the prevalence of virulence genes between the invasive and non-invasive infection groups, we found significantly higher detection rates of *fyuA*, K1, *ibeA*, and *iroN* in the invasive group. Among them, *fyuA* and *iroN* are both iron acquisition-related virulence genes [28]. Iron acquisition systems are commonly associated with ExPEC strains isolated from patients with bacteremia and are involved in the efficient uptake of iron from the blood as well as bacterial invasion of the bloodstream from the urinary tract [29,30]. The K1 capsule has been linked to neonatal sepsis and bacterial meningitis, and also correlates with disease severity [31,32]. Studies have shown that K1 capsule was present in 50 % of isolates from neonatal sepsis and 80 % of isolates from neonatal meningitis [33,34]. The K1 capsule is composed of polysialic acid (polySia), that mimics the polySia modification found on human neuronal and immune cells and likely promotes the capacity of K1-expressing *E. coli* to hide and reside within the blood and neuronal compartments [35]. Indeed, polySia prevents the full activation of host innate defenses and confers resistance to complement- and phagocyte-mediated killing [36]. Consistent with validation in animal models, K1 expression promotes the development of invasive infections by *E. coli* [37]. Regarding IbeA proteins, it has been suggested that Caspr1, a membrane protein expressed on HBMEC, acts as a receptor for IbeA to facilitate blood–brain barrier penetration and entry of *E. coli* into brain neurons via ligand-receptor interactions [38].

In our study, the invasive infection group contained 11 cerebrospinal fluid specimens, with 90.9 % (10/11) K1 detection rate and 36.4 % (4/11) *ibeA* detection rate, which further validated that K1 capsule and IbeA proteins assisted *E. coli* to cross the blood-brain barrier (BBB). Our findings suggest that *fyuA*, K1, *ibeA*, and *iroN* may be the key virulence genes that determine the development of invasive infectious events such as neonatal sepsis and meningitis.

In addition to virulence genes prevalence data, our study also provides information on the AMR of neonatal *E. coli* strains. A substantial proportion of neonatal *E. coli* isolates were resistant to commonly administered antibiotics, with cefotaxime showing the

highest rate of resistance (75.5 %, 120/159), and 98 (61.6 %) isolates were MDR. Although this may be affected by differences in sample isolation sites, the antibiotic resistance situation in China is more severe than that in developed regions such as the United States and Europe [23,39]. A particular concern is the discovery of a strain with a colistin-resistant phenotype in this study. Colistin is considered among the last resort for treating MDR *Enterobacteriaceaea*, and the emergence of its resistance could pose a severe threat to the healthcare system [40].

E. coli antibiotic resistance has become a global challenge, and although virulence and resistance develop at different times, they are not independent characteristics; rather, there is a relationship between them [13]. We analyzed the correlation between virulence genes and AMR using epidemiological data. Overall, the number of virulence genes carried by each *E. coli* strain positively correlated with the number of resistant antibiotics, which possibly reflects the evolutionary trend of clonal populations combining resistance and virulence, such as ST131 [41]. In this study, the strongest positive correlation was observed in *kpsMT* III & ciprofloxacin. *KpsMT* III encodes group III capsules (K10 and K54) [42], which are protective structures on the surfaces of bacteria [27]. It is not surprising to find elevated resistance in *kpsMT* III-positive strains; however, there is very little information available about group III capsules, and the specific molecular mechanisms require further exploration.

Notably, the detection of *kpsMT* III and *fimH* was associated with increased quinolone resistance, whereas the detection of *traT* and *papA* was associated with decreased quinolone resistance. Resistance to quinolones is largely mediated by point mutations in DNA gyrases and topoisomerases, but it may also be due to the synergistic combination of efflux pumps and plasmid-mediated mechanisms [43]. Despite repeated attempts at explaining the biology of the inverse link between quinolone-resistance and virulence, the conflicting results found in the literature underlie the complexity of the topic [44]. Typically, ExPEC-associated virulence, such as P fimbriae (pap) and haemolysin (hly), have been found to be less common in the genomes of quinolone-resistant isolates. This finding may be explained by the acquisition of quinolone resistance by *E. coli* naturally lacking these virulence genes, subsequently spread in a clonal fashion [45]. Another hypothesis is that during the development of quinolone resistance, antibiotics can increase the deletion or translocation of some DNA regions containing virulence genes, or partial or total loss of PAI due to possible inhibition of topoisomerases II and IV [46,47]. There are positive and negative correlations between antibiotic resistance and virulence, depending on the antibiotic studied, the mechanism of resistance and the type of *E. coli* [12]. The mechanisms involved in the relationship between resistance and virulence are complex, and epidemiological associations are only the first step to understanding them. Therefore, more in-depth molecular studies of virulence genes and AMR determinants at the genetic level are urgently needed to explore the interactions and co-evolutionary paths between virulence and resistance.

The strength of our study is that the samples were obtained from multiple medical centers across China, and thus, our findings provide specific information on virulence genes and AMR in neonatal *E. coli* infections across Chinese populations. However, this study has several limitations that should be noted when interpreting our findings. First, we examined only virulence genes and antimicrobial susceptibility in *E. coli*, and our findings lack further patient categorization, clinical symptom, and diagnostic information. Records on antibiotic dose and administration frequency and detailed patient-level data were not recorded nor included in the analyses. Second, we acknowledge that the patients included in this study were from tertiary hospitals and their condition might be more severe than that of sick infants in primary medical centers.

In conclusion, we have characterized in detail the virulence and AMR of *E. coli* isolates from Chinese hospitalized neonates. We found that neonatal *E. coli* isolates carried several virulence genes and were severely resistant to commonly administered antibiotics. We also showed a higher prevalence of *fyuA*, K1, *ibeA* and *iroN* in *E. coli* causing invasive infections. The presence of these virulence genes may predict the site of infection and severity of disease. This knowledge can be used for the molecular detection of virulent strains, and could also be targets for the development of new therapeutic strategies, such as vaccines, in the era of antimicrobial resistance. A correlation between virulence genes and AMR was observed in this work. Further studies will be needed to understand the molecular mechanisms underlying the correlation between virulence and AMR.

5. Transparency declaration

The authors declare that they have no conflicts of interest. This work was funded by the National Natural Science Foundation of China (Grant No. 81872676) and Beijing Natural Science Foundation (Grant No. 7232009).

Data Availability statement

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Yajuan Wang cxswyj@vip.sina.com. The data reported in this paper may be shared by the lead contact on request and pending approval from ethics and regulatory committees where relevant. This study didn't report original code. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

Ethics declarations

This study was reviewed and approved by Beijing Children's Hospital with the approval number: No. 2019-k-350, dated Oct 7, 2019.

CRediT authorship contribution statement

Yuting Guo: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ruiqi Xiao:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Jinxing Feng:** Writing – review & editing, Investigation, Data curation. **Xiaoyun Wang:** Writing – review & editing, Resources, Methodology, Investigation, Data curation. **Jidong Lai:** Resources, Methodology, Investigation. **Wenqing Kang:** Resources, Investigation. **Yangfang Li:** Resources, Investigation. **Xueping Zhu:** Resources, Investigation. **Tongzhen Ji:** Resources, Investigation. **Xuerong Huang:** Resources, Investigation. **Dan Pang:** Resources, Investigation. **Yanbin An:** Resources, Investigation. **Lihui Meng:** Writing – review & editing, Project administration, Conceptualization. **Yajuan Wang:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e35991.

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