Characteristics of a capnophilic small colony variant of *Escherichia coli* co-isolated with two other strains from a patient with bacteremia in China

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Abstract
Small colony variants (SCVs) are a slow-growing subpopulation of bacteria characterized by their atypical colony morphology and distinct biochemical properties, which are known to cause chronic persistent infections. Here, we investigated the characteristics of three phenotypes of *Escherichia coli*, including a capnophilic SCV, co-isolated from a 64-year-old patient with bacteremia in China. The three strains were identified as a capnophilic strain (EC1), a capnophilic SCV (EC2), and a normal colony strain (EC3). The EC1 and EC2 strains did not grow in the absence of CO2, while the EC2 colonies were pinpoint in appearance and had the ability to revert to the normal colony phenotype. The growth of the SCV was slow and not enhanced in the presence of thymidine, hemin, thiamine, and menadione. The results of antimicrobial susceptibility among the three strains showed similar sensitivity to cefoxitin and imipenem, but resistant to most of the other antimicrobials tested. Whole-genome sequencing showed that no genetic mutational variations associated with SCVs were observed, while EC1, EC2 and the revertible strains of EC2 lacked the *can* gene. Multi-locus sequence typing showed that all strains belonged to ST457 and nucleotide similarity analysis indicated that they had high homology. In conclusion, we report rarely described co-isolated forms of three phenotypes of *E. coli* that included a capnophilic SCV in a patient with bacteremia. The capnophilic SCV strain had atypical morphology and biochemical characteristics in the absence of *can* gene. Based on our findings, we have discussed the laboratory identification, characterization, mechanisms, and clinical treatment of capnophilic SCV strains.

Keywords *Escherichia coli* · Small colony variants · Capnophilic phenotype · Complete genome sequence · Bacteremia

Abbreviations

| Abbreviation | Description |
|--------------|-------------|
| SCV          | Small colony variants |
| *E. coli*    | *Escherichia coli* |
| WGS          | Whole-genome sequencing |
| BAP          | Blood agar plate |
| MALDI-TOF MS | Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry |
| MH           | Mueller Hinton |
| MIC          | Minimum inhibitory concentration |
| MLST         | Multi-locus sequence typing |
| SNP          | Single nucleotide polymorphism |

Introduction
Bacteria are capable of adapting to environmental pressures through changes in their phenotype and genetics. Small colony variants (SCVs) constitute a naturally occurring sub-population of bacteria with atypical colony morphology and unusual biochemical characteristics. The most conspicuous feature of SCVs is the small size of the colonies compared with wild-type bacteria, the slow growth rate, and low susceptibility to antibiotics, which pose a challenge for clinical identification and treatment (Proctor et al. 2006). At present, SCVs have been identified in several genera of bacteria (Swingle 1935; Bryan and Kwan 1981; Mowjood et al. 1979). The greatest amount of information about SCVs has been obtained from
Staphylococcus aureus, while there have been very few reports about SCVs in clinical specimens of Escherichia coli (Roggengeance et al. 1998; Tappe et al. 2006; Negishi et al. 2018).

Most SCVs that have been recovered so far are deficient in electron transport and thymidine biosynthesis, and these phenotypes can be reversed by supplementation with menadione, hemin, or thymidine (Proctor et al. 2006). E. coli SCVs with hemB, hemD, hemL or lipA mutations have been used for functional characterization of changes in growth, metabolism, antimicrobial susceptibility, and persistence (Ramiro et al. 2016; Santos and Hirshfield 2016). However, capnophilic E. coli SCVs have rarely been reported. The first case of bacteremia by capnophilic E. coli showed genetic loss of the YaqD gene (Sahuquillo-Arce et al. 2017), and in 2018, co-existence of E. coli SCVs and E. coli with capnophilic phenotypes was reported in a patient with urinary tract infection (Park et al. 2018). However, the genetic and biochemical basis of E. coli SCVs and capnophilic E. coli emergence in the in vivo context is not well understood.

With regard to their clinical characteristics, SCVs are better able to persist in cells and are less susceptible to antibiotics than their wild-type species (Proctor et al. 2006). These characteristics make their clinical identification difficult and lead to persistent or recurrent infection on emergence from the protective environment of the host cell. From the clinical microbiology perspective, it has been difficult to identify and assess the antimicrobial susceptibility of SCVs due to a lack of established standards. Moreover, the incidence of SCVs in clinical specimens may be underestimated, while some more patients who suffer from persistent infection may be caused by SCVs. Accordingly, it is important to focus on the monitoring and detection of SCVs with comprehensive studies on the phenotypic and genetic characterization of E. coli SCVs.

In this study, we report three different phenotypic strains of E. coli, including a normal colony phenotype, a capnophilic strain and a capnophilic SCV form, which were co-isolated from a patient with bacteremia in China. We examined the phenotypic features, laboratory diagnosis, and antimicrobial sensitivity of the three strains. In addition, whole-genome sequencing (WGS) was performed to determine the causal genetic mechanisms that distinguish the capnophilic E. coli and capnophilic SCV from the wild-type strain. The results provide important evidence for the identification, mechanisms, and clinical treatment of capnophilic SCVs.

Materials and methods

Clinical features of the patient

A 64-year-old woman suffered from kidney stones and hydrenephrosis was admitted to Nanjing Drum Tower Hospital on April 22, 2018. After percutaneous nephrolithotomy on April 24, the patient had a high fever with a body temperature of 40.7 °C and signs of septic shock. Her white blood cell count was 15.8 x 10^9/L; neutrophil percentage, 97.1%; C-reactive protein level, 65.9 mg/L; and procalcitonin, 4.94 ng/mL. The patient was transferred to the ICU for replenishment of blood volume by treatment with a vascular active medicine, and also received oxygen inhalation treatment. For anti-infection therapy, the patient was treated with imipenem (500 mg, Q8H) for one week. Based on elevated blood creatinine level (136 mmol/L) and oliguria, acute renal injury was diagnosed and the patient received renal replacement therapy. The patient recovered well and was discharged on May 9. The study protocol was approved by the Ethics Committee of Nanjing Drum Tower Hospital of Nanjing University Medical School, and the ethical approval number was 2022-CR001-01.

Bacterial culture and isolation

A urine sample and two sets of blood samples were cultured for bacterial identification. Gram-negative bacilli were identified on microscopic examination after 18-h culture of the blood samples in aerobic and anaerobic bottles. The samples were also cultured on a sheep blood agar plate (BAP) at 35 °C in a 5% CO2 atmosphere overnight, and a slow-growing, needle-tip-like small colony was identified on the BAP. We named the three strains of Gram-negative colonies isolated from blood culture as EC1, EC2, and EC3. EC1 and the needle-tip-like EC2 were isolated from the same aerobic culture bottle, while EC3 was isolated from the anaerobic culture bottle. The urine sample was cultured on BAP at 35 °C in a 5% CO2 atmosphere. After 24 h of incubation, > 10,000 cfu/mL of Gram-negative colonies were detected on the BAP. The strains identified in the blood and urine samples were isolated for further characterization.

Colony morphology analysis and Gram staining

The morphological characteristics of strains EC1, EC2, and EC3 were compared in three different culture conditions: aerobic, 5% CO2, and anaerobic. We also observed the morphological characteristics of the strains at three-time points: after 24, 48, and 72 h of culture. The EC1, EC2, and EC3 bacterial cells were also analyzed by Gram staining. E. coli ATCC 25922 was used as the control strain.

Identification of strains

The three strains, EC1, EC2, and EC3, were identified using the VITEK 2 system (bioMérieux, France) and the API 32E system (bioMérieux, France) according to the manufacturer’s instructions. Matrix-assisted laser desorption/ionization
time-of-flight mass spectrometry (MALDI-TOF MS) (micro Typer MS) was used to confirm the identification. The colonies isolated from urine were identified by the VITEK 2 system. The biochemical characteristics of the strains were examined with the oxidation fermentation experiment and oxidase test.

**Antimicrobial susceptibility testing**

Antimicrobial susceptibility testing was performed using the VITEK 2 system with the GN13 card and the disk diffusion method (Oxoid Lid, UK). For the disk diffusion method, a 0.5 McFarland suspension of each of the three strains was prepared and spread on Mueller Hinton (MH) agar and cultured in a 5% CO₂ environment for 16–18 h with the following antimicrobials: ampicillin, ampicillin-sulbactam, amikacin, ceftazidime, cefotaxime, cefuroxime, cefoxitin, imipenem, piperacillin-tazobactam, cefepime, levofloxacin, and sulfamethoxazole-trimethoprim. The minimum inhibitory concentration (MIC) and disk diffusion method results were determined based on the criteria for testing Enterobacterales according to CLSI M100 (Clinical and Laboratory Standards Institute 2018).

**Auxotrophy testing**

Auxotrophy tests for thymidine, hemin, thiamine, and menadione were performed as described previously (Wellinghausen et al. 2009). We carried out the auxotrophy testing by gradient concentration as 10, 20, 50, and 100 µg/mL of each supplement, and the final concentration of the four supplements was 20 µg/mL in MH agar. EC1, EC2, EC3, and ATCC25922 were inoculated on the MH agar and incubated at 35 °C in 5% CO₂ or aerobic conditions for 48 h.

**Preparation of genomic DNA and WGS analysis**

Genomic DNA of the three strains was extracted from a culture grown overnight in Luria-Bertani medium at 35 °C under agitation (220 rpm) using the QIAamp DNA mini-kit (Qiagen, Hilden, Germany). Library preparations were constructed following the manufacturer’s protocol (Illumina TruSeq DNA Nano Library Prep Kit) and sequenced using the IlluminaHiseq PE150 platform to generate 450-bp paired-end reads. Trimmed sequencing reads were subjected to de novo assembly with SPAdes pipeline version 3.9.0 (Bankevich et al. 2012) under the default settings. Multi-locus sequence typing (MLST) was performed according to a previously published method (Larsen et al. 2012). WGS was performed to identify genes that contained previously reported causative mutations for the *E. coli* SCV phenotypes and the nucleotide similarity was analyzed by CLC Genomics Workbench 21.0.3. The genome sequences of EC1, EC2, and EC3 have been deposited in GenBank under accession numbers QVHR00000000, QVHQ00000000, and QVHS00000000, respectively.

**PCR analysis**

Extracted DNA was amplified by PCR under standard conditions as described previously (Sahuquillo-Arce et al. 2017) with the following primers for amplification of the 510-bp fragment of the *can* gene: *canF* (5′-TTGGTGCGGTGAACTCCAG-3′) and *canR* (5′-GAAACTGGCACAAGCGCAAAA-3′). The following PCR protocol was used: 5 min at 94 °C; 35 cycles of 1 min at 94 °C, 1 min at 55 °C, and 1 min at 72 °C; and a final extension of 7 min at 72 °C. The amplification products were separated by electrophoresis on an agarose gel with 2% ethidium bromide staining, and the size of the amplicons was determined based on their homology with a molecular weight marker (PCR marker; Promega Corporation, Madison, WI, USA) DL2000.

**Results**

**Colony and cell morphologies of the three co-isolated strains**

After 48 h of incubation on BAP at 35 °C in a 5% CO₂ atmosphere, the EC1 and EC3 colonies appeared sleek and greyish, but the EC1 colony appeared slightly drier than the EC3 colony. In contrast, the EC2 colonies had a needle-tip-like appearance and were dry and not easy to pick from the BAP surface (Fig. 1a). Gram staining showed that EC1 and EC2 were heterogeneous, swollen, and elongated, and most of the EC3 cells were of the same size as *E. coli* ATCC25922 cells (Fig. 1b).

**Growth characteristics of strains under different conditions**

We compared the morphology of the three colonies in different culture environments (aerobic, 5% CO₂, and anaerobic) and at different time points (24, 48, and 72 h) (Fig. 2). EC1 and EC2 failed to grow under aerobic conditions in the absence of CO₂ and EC3 showed growth in this condition. Under the 5% CO₂ condition, EC2 colonies showed slow growth and tightly adhered to the BAP. All three isolates grew in the anaerobic environment but appeared paler than they did in the CO₂ environment (Fig. 2). Notably, EC2 exhibited the ability to revert to the large-form EC1 phenotype in the presence of CO₂ during passage culture after 6 generations, and did not demonstrate growth defects compared to EC1 and EC3 under anaerobic conditions (Fig. 2).
Differences in biochemical characteristics

Biochemical identification with the VITEK 2 system and API 32E system identified all three colonies as *E. coli*, and this was confirmed by MALDI-TOF MS. In contrast to EC3 (which was identified as a wild-type strain), EC1 and EC2 did not ferment glucose or express tyrosine arylaminase. EC2 was positive for oxidase and the others were negative (Table 1). The strain isolated from the urine sample was identified as *E. coli* and had the same biochemical characteristics as the wild-type EC3 strain.

Antimicrobial susceptibility results

The capnophilic EC1 and EC2 strains could not be cultured in ambient air with the VITEK 2 system, which showed terminated results of antimicrobial susceptibility in the system. The disk diffusion method under 5% CO₂ conditions showed that EC1, EC2, and EC3 were resistant to ampicillin, ampicillin-sulbactam, amikacin, levofloxacin, and sulfamethoxazole-trimethoprim, and they were susceptible to cefoxitin and imipenem (Table 2). Additionally, EC1 and EC2 were resistant to ceftazidime and cefepime, while EC3 exhibited...
intermediate resistance to ceftazidime and cefepime. Further, EC2 and EC3 were susceptible to piperacillin-tazobactam, while EC1 showed intermediate susceptibility. The E. coli strain isolated from the urine sample had similar antimicrobial susceptibility features with EC3.

**Auxotrophic features of EC1, EC2, and EC3**

After culturing for 48 h in 5% CO₂ conditions, the growth of EC2 on MH agar containing thymidine, hemin, thiamine, and menadione was not enhanced. Under aerobic conditions, EC1 and EC2 were unable to grow in the presence of thymidine, hemin, thiamine, and menadione except EC3 and ATCC25922 (Fig. 3).

**WGS analysis**

The results of WGS of the three isolated E. coli strains were highly consistent (Table 3). A set of 6 virulence genes and 13 resistance genes were identified in each strain with the VirulenceFinder (2.0) and ResFinder (3.2) servers of the Center for Genome Epidemiology website. MLST results showed that all three strains belong to ST457 (MLST 2.0, http://www.genomicepidemiology.org). Nucleotide similarity analysis indicated that the three strains had high homology (>99%). With regard to single nucleotide polymorphism (SNP), only 4 high-quality SNPs were detected between EC1 and EC2. These SNPs located in (G > C, 294,872, DZA82_01605), (A > C, 116,322, DZA82_04645), (A > G, 42,425, DZC26_11995), (C > T, 182,874, DZC26_12685), and the corresponding coding products were 2-oxoglutarate dehydrogenase subunit E1, LLM class flavin-dependent oxidoreductase, insulinase family protein and TetR/AcrR family transcriptional regulator, respectively. There were

### Table 1 Differences in biochemical reactions of E. coli strains

| Biochemical reaction | EC1 | EC2 | EC3 |
|----------------------|-----|-----|-----|
| BGAL                 | +   | +   | +   |
| H2S                  | −   | −   | −   |
| dGLU                 | +   | +   | +   |
| O-F                  | −   | −   | +   |
| dMAL                 | +   | +   | +   |
| TyrA                 | −   | −   | +   |
| URE                  | −   | −   | −   |
| SAC                  | +   | −   | +   |
| dTAG                 | −   | −   | +   |
| MNT                  | −   | −   | −   |
| PHOS                 | −   | −   | +   |
| ODC                  | −   | −   | −   |
| O129R                | −   | −   | +   |
| OX                   | −   | +   | −   |
| Catalase             | +   | +   | +   |
| IND                  | +   | +   | +   |

*BGAL* β-galactosidase, *dGLU* D-glucose, *H2S* H₂S H₂S production, *O-F* oxidative-fermentation test, *dMAL* D-maltose, *dMAN* D-mannitol, *TyrA* tyrosine arylamidase, *URE* urease, *SAC* saccharose, *dTAG* D-tagatose, *MNT* malonate, *PHOS* phosphatase, *ODC* ornithine decarboxylase, *O129R* 2,4-miamino-6,7-diisopropylpterdine, *OX* oxidase, *IND* indole

### Table 2 Antimicrobial susceptibility results of E. coli strains

| Antimicrobial agent       | Disk diffusion method (5% CO₂) | VITEK 2 system GN13 card |
|---------------------------|--------------------------------|------------------------|
|                           | EC1   | EC2   | EC3   | EC1 | EC2 | EC3 (MIC) |
| Ampicillin                | 6(R)  | 6(R)  | 6(R)  | Terminated | Terminated | > = 32(R) |
| Ampicillin-sulbactam      | 8(R)  | 6(R)  | 8(R)  | > = 32(R)  | > = 32(R)  |
| Amikacin                  | 6(R)  | 6(R)  | 6(R)  | > = 64(R)  | > = 64(R)  |
| Ceftazidime               | 17(R) | 15(R) | 18(I) | 8(I)   |           |           |
| Cefotaxime                | 6(R)  | 6(R)  | 6(R)  |       |           |           |
| Cefuroxime                | 6(R)  | 6(R)  | 6(R)  |       |           |           |
| Cefoxitin                 | 20(S) | 25(S) | 20(S) | < = 4(S) | < = 4(S)  |           |
| Imipenem                  | 27(S) | 30(S) | 26(S) | < = 1(S) | < = 1(S)  |           |
| Piperacillin-tazobactam   | 20I   | 26S   | 22S   | < = 4(S) | < = 4(S)  |           |
| Cefepime                  | 6(R)  | 6(R)  | 15(I) | 4(I)   |           |           |
| Levofloxacin              | 6(R)  | 6(R)  | 6(R)  | > = 8(R) | > = 8(R)  |           |
| SMZ-TMP                   | 6(R)  | 6(R)  | 6(R)  | > = 320(R)| > = 320(R)|           |

*S* sensitive, *I* intermediate, *R* resistant
34 high-quality SNPs detected between EC1 and EC3. However, no genetic mutational variations associated with SCVs (in the hemA, hemB, hemL, hemY, yigP, nuoG, thyA, and lipA genes) were observed in the three strains. The can gene, which is absent in capnophilic E. coli strains, was not present in EC1 and EC2 but expressed in EC3. The PCR results also confirmed that the can gene was present in EC3, ATCC25922 E. coli and clinical isolates of E. coli, but absent in EC1, EC2, the revertible isolate of EC2 and the clinical isolates of Klebsiella pneumoniae and Pseudomonas aeruginosa (Fig. 4).

Discussion

SCVs are usually associated with chronic persistent infection. There are very few reports about clinical specimens of E. coli SCVs that cause urinary tract infection, prosthesis-related infection, and bacteremia. From 1977 to 1978, Borderon and Horodniceanu (1978) observed 16 small colony mutants from 745 strains of E. coli isolated from urine that caused pyuria and persistent urinary tract infection. In 2005, a case of febrile bacteremia due to an E. coli SCV was reported, in which the patient also experienced a long-term urinary tract infection (Tappe et al. 2006). Additionally, two studies reported that E. coli SCVs were responsible for infection of the prosthesis joint, which is a serious complication that occurs after joint replacement (Roggenkamp et al. 1998; Sendi et al. 2010). In 2018, co-existing E. coli SCVs and capnophilic phenotypes isolated from a patient with urinary tract infection were first reported (Park et al. 2018). However, we believe that the actual clinical prevalence of infections caused by E. coli SCVs is far greater than the reported incidence so far. In the present study, we describe three different phenotypic strains of E. coli, including a wild-type strain, a capnophilic strain, and a capnophilic SCV, that rarely co-existed in a patient with kidney stone-associated infection and bacteremia. The capnophilic strain and capnophilic SCV that were isolated from her blood sample had different growth and biochemical features, while
WGS analysis indicated that the three different phenotype strains were highly consistent.

Mutations of the gene encoding the key enzymes in the electron transfer system and thymidylate synthase are responsible for the emergence of SCVs. In addition, biofilm and antibiotic pressure also promote such variations. In 1998, Roggenkamp et al. (1998) reported the small colony mutant hemB E. coli in a case of artificial hip joint infection for the first time. Further, according to a recent report, a novel SNP in hemA of a clinical isolate of E. coli was responsible for an SCV phenotype (Hubbard et al. 2021). HemA is a glutamyl-tRNA reductase that catalyses the initial step of porphyrin biosynthesis leading to the production of haem and when supplied hemin exogenously during growth of SCV on agar could reverse the SCV phenotype to normal size. With regard to thymidine-dependent SCVs of E. coli, mutation in the thyA gene (c.62G > A) was found to cause morphological abnormalities in the colonies and thymidine auxotrophy (Negishi et al. 2018). Recent studies also showed that mutation in the lipoic acid synthase gene (lipA) resulted in SCV phenotypes and slow growth of E. coli bw25113. Additionally, the expression of the biofilm genes wcaC and wcaK in the lipA mutant strain was also higher than that in the wild-type strain (Santos and Hirschfield 2016). In our report, the SCV EC2 did not exhibit thymidine auxotrophy or electron transport defects. Further, EC2 had the ability to revert to the large-form EC1 phenotype in the presence of CO₂ during passage culture after 6 generations, and the reverted colonies also exhibited a capnophilic phenotype (Fig. 2). This indicates a potential in vivo survival mechanism of E. coli SCVs, which was similar to Enterobactericeae reported by Greninger et al. (2021). No genetic mutations associated with the E. coli SCV phenotype were detected in the hemA, hemB, nuoG, thyA, lipA and other genes including ubiE, acnA, fdhG, pta and crcB in the three strains. Capnophilic E. coli have rarely been reported (Matsumoto et al. 2020; Lu et al. 2012; Tena et al. 2008), so our strains make an important contribution to the database of capnophilic E. coli. The can gene was not detected in EC1 and EC2, which was consistent with previous reports on capnophilic E. coli (Sahuquillo-Arce et al. 2017; Park et al. 2018). Carbonic anhydrase activity is supplied by the product of can which is essential for aerobic growth of E. coli, and the impaired carbonic anhydrase function can cause a carbon dioxide-dependent SCV phenotype in E. coli (Matsumoto et al. 2020). The nucleotide similarity analysis indicated that the three strains had high homology, and the WGS data showed highly consistent in virulence and resistance genes between the three stains. Comparison study for the SNPs between the three strains revealed that some differential SNPs may act in catalyzing the oxidative decarboxylation of 2-oxoglutarate to succinyl-CoA and NADH (Schulze et al. 1990), while TetRs act as chemical sensors to monitor both the cellular environmental dynamics and their regulated genes underlying many events, such as antibiotics production, osmotic stress, efflux pumps, multidrug resistance, metabolic modulation, and pathogenesis (Deng et al. 2013). Even some SNPs were found between EC1, EC2 and EC3, the function of the differential SNPs and the mechanism of action in the strain variants still need to be further studied. Further research on the transcription level and protein level of these strains could shed light on the mechanism of the emergence of capnophilic SCVs.

The identification and treatment of SCV infections have posed a challenge to microbiologists and clinicians. In addition to their atypical colony morphology, they also have a deficiency in biochemical reactions. Therefore, it is difficult to identify them based on their biochemical profile. Genetic profiling with methods such as 16S rRNA sequencing, MALDI-TOF MS, and WGS are of great value in the identification and characterization of SCVs. To reduce the possibility of missing CO₂-dependent SCVs, the microbial samples should be cultured in a 5% CO₂ environment for a prolonged time. Due to the slow growth and low metabolic

| Strains | Contigs | Genes | Proteins | rRNA | tRNA | ncRNA | Bases | N50 | MLST | Plasmids | Virulence genes | Resistance genes |
|---------|---------|-------|----------|------|------|-------|-------|-----|------|---------|----------------|----------------|
| EC1     | 125     | 5326  | 4984     | 12   | 82   | 7     | 5,130,567 | 242,130 | ST457 | IncX1   | eilA, lipA, air, iss, gad |
| EC2     | 124     | 5342  | 4999     | 12   | 83   | 7     | 5,146,176 | 241,168 | p0111 |                      |
| EC3     | 120     | 5343  | 4999     | 12   | 82   | 7     | 5,145,687 | 241,168 |        |                      |

Table 3 Information of whole-genome sequencing of E. coli strains
rate of SCVs, conventional antimicrobial susceptibility tests, including the disk diffusion method, E-test, micro-dilution tests, and automated susceptibility testing systems, may result in errors (Proctor et al. 2006). The antimicrobial susceptibility results in our report were not significantly different when all strains were tested with a same 5% CO₂ environment culture. Therefore, for antimicrobial susceptibility testing of SCVs, it is necessary to ensure that the bacteria are cultured for a sufficient amount of time on the plate or in the liquid according to colony growth, and the MIC values must be carefully evaluated. The most suitable methods for antimicrobial susceptibility testing of E. coli SCVs should be further explored. For antibiotic therapy in the clinical setting, all the co-existing strains and SCVs must be considered. Secondly, it is important to avoid certain antibacterial drugs, such as aminoglycosides, based on the auxotrophic features of SCVs, as some findings have shown that SCVs of E. coli are resistant to aminoglycoside antibiotics (Lewis et al. 1991). In our study, there was no significant difference in the drug sensitivity of the three strains of E. coli, but they were all resistant to aminoglycosides. The patient was treated with imipenem successfully and was discharged soon.

Conclusions

We report three different phenotypic strains of E. coli, including a capnophilic strain, a capnophilic SCV and a normal colony phenotype that were co-isolated from a patient with bacteremia. The three phenotypes of E. coli showed high homology in nucleotide level, and capnophilic SCV strain had atypical morphology and biochemical characteristics in the absence of can gene. The phenotypic changes, production mechanism, laboratory identification, and clinical treatment of SCVs were also discussed. Although the mechanism for the formation of E. coli SCVs in clinical has not been clarified, our study still shed more light on bacterial metabolism, signaling, and virulence for E. coli SCVs. Importantly, more research on these SCVs could improve their diagnosis and monitoring, as well as the treatment and outcome of persistent infection caused by SCVs.

Author contributions GS, ZW and SH performed design, contributed to data collection and drafted the manuscript. ZZ and XX performed the culture and molecular experiment. ZH and ZH were responsible for the identification and antimicrobial susceptibility test. ZY was responsible for editing the figures. CX performed WGS and analyzed the data. All authors have read and approved the final manuscript.

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Availability of data and material Whole-genome sequencing data are available in GenBank with the accession number QVHR00000000, QVHQ00000000 and QVHS00000000, respectively.

Code availability Not applicable.

Declarations

Conflicts of interest/Competing interests All authors declare that they have no conflict of interest.

Ethics approval The study protocol was approved by the Ethics Committee of Nanjing Drum Tower Hospital of Nanjing University Medical School (approval number: 2022-CR001-01).

Informed consent Written informed consent was obtained from the patient for the publication of this paper.

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