Antimicrobial Resistance and Infection Control

CASE REPORT

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Urinary tract infection caused by a small colony variant form of capnophilic *Escherichia coli* leading to misidentification and non-reactions in antimicrobial susceptibility tests

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Abstract

Background: Small colony and capnophilic variant cases have been separately reported, but there has been no reports of their simultaneous presence in one isolate. We report a case of *Escherichia coli* with coexpressed small colony and capnophilic phenotypes causing misidentification in automated biochemical kits and non-reactions in antimicrobial susceptibility test cards.

Case presentation: An 86-year-old woman developed urinary tract infection from a strain of *Escherichia coli* with SCV and capnophilic phenotypes in co-existence. This strain did not grow without the presence of CO_2, and therefore proper identification from automated system was not possible. 16 s rRNA sequencing and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry was able to identify the bacteria.

Conclusion: As these strains do not grow on culture parameters defined by CLSI or on automated systems, proper identification using alternative methods are necessary.

Keywords: Small colony variant, Capnophilic, *Escherichia coli*, Misidentification

Background

Small colony variants (SCV) can be defined as a naturally occurring sub-population of bacteria characterized by their reduced colony size and distinct biochemical properties [1]. Capnophilic *E. coli*, which thrive in the presence of high concentrations of carbon dioxide, have rarely been reported [2, 3]. SCV and capnophilic variant cases have never been reported in co-existence. Herein, we report the first case of *E. coli* with coexpressed SCV and capnophilic phenotypes isolated from a urinary tract infection.

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Case report

An 86-year-old woman visited our hospital with foamy urine and foul odor. Urinalysis showed many WBCs (163.7 WBCs/μL) and bacteria (11,343.7 bacteria/uL), and positivity for nitrite. Gram-negative coccobacilli were revealed upon microscopic examination. The sample was cultured on sheep blood agar plate (BAP) and MacConkey agar plates at 35 °C in a 5% CO_2 atmosphere for 24 h. After one day of incubation, > 100,000 CFU/ml of pinpoint Gram-negative colonies grew on the BAP with 10,000 CFU/ml of Gram-positive cocci. After isolation of pinpoint colonies and another 24-h incubation, the pinpoint Gram-negative colonies were irregularly divided into large colonies and pinpoint SCV colonies on BAP (Table 1).

While the VITEK 2 system (bioMerieux, Durham, USA) identified the pinpoint colony as *Burkholderia cepacia* group, the Bruker Biotyper (Bruker Daltonics, Leipzig,

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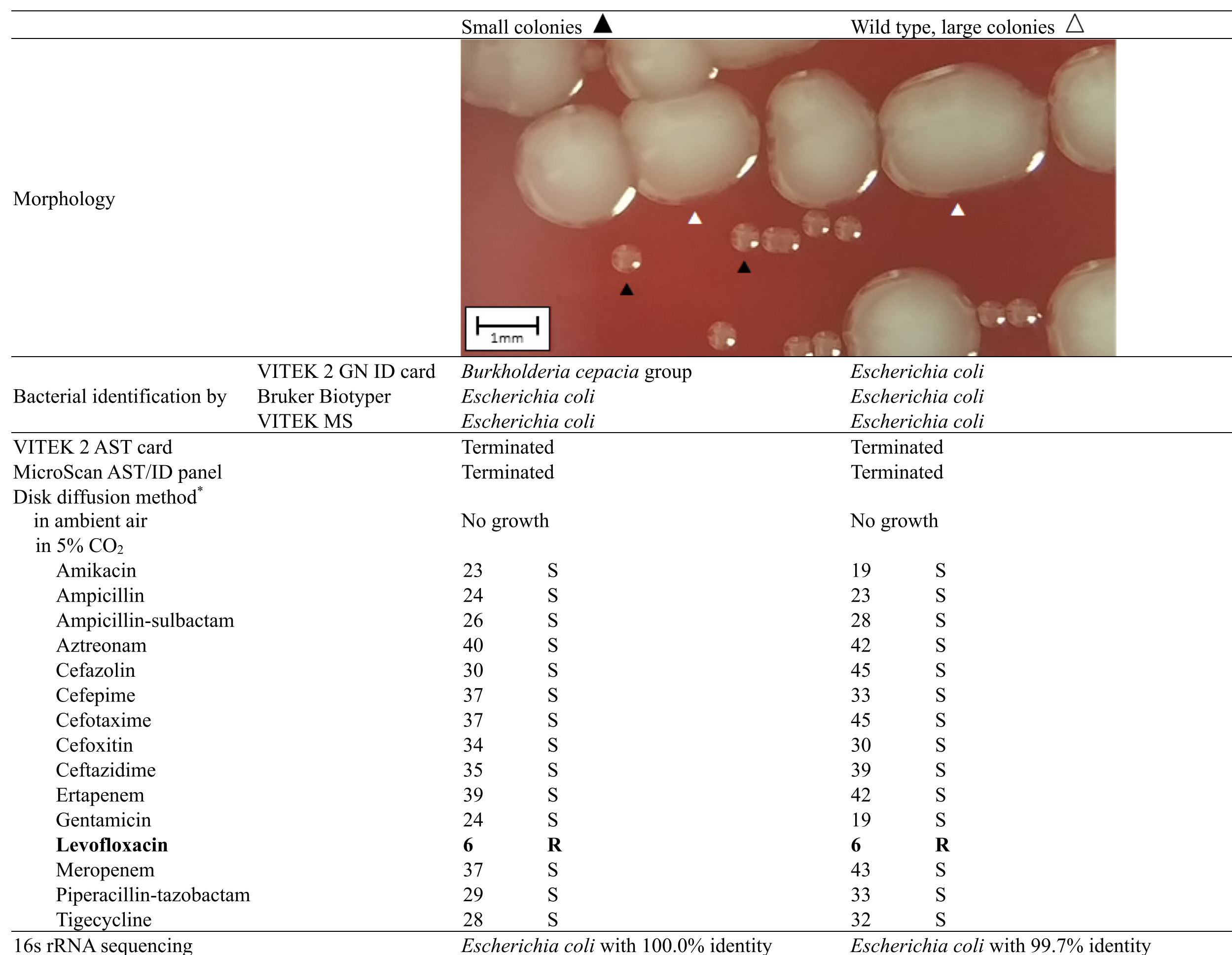
Table 1 Bacterial identification and antimicrobial susceptibility testing results

		Small colonies ▲		Wild type, large colonies △	
Morphology					
Bacterial identification by	VITEK 2 GN ID card	*Burkholderia cepacia* group		*Escherichia coli*	
	Bruker Biotyper	*Escherichia coli*		*Escherichia coli*	
	VITEK MS	*Escherichia coli*		*Escherichia coli*	
VITEK 2 AST card		Terminated		Terminated	
MicroScan AST/ID panel		Terminated		Terminated	
Disk diffusion method*					
in ambient air		No growth		No growth	
in 5% CO_2					
Amikacin		23	S	19	S
Ampicillin		24	S	23	S
Ampicillin-sulbactam		26	S	28	S
Aztreonam		40	S	42	S
Cefazolin		30	S	45	S
Cefepime		37	S	33	S
Cefotaxime		37	S	45	S
Cefoxitin		34	S	30	S
Ceftazidime		35	S	39	S
Ertapenem		39	S	42	S
Gentamicin		24	S	19	S
Levofloxacin		**6**	**R**	**6**	**R**
Meropenem		37	S	43	S
Piperacillin-tazobactam		29	S	33	S
Tigecycline		28	S	32	S
16s rRNA sequencing		*Escherichia coli* with 100.0% identity		*Escherichia coli* with 99.7% identity	

*Disk diffusion method results are given as measured zone diameters [8] and interpretive category. *S* susceptible, *R* resistant

Germany) and VITEK MS (bioMerieux, Marcy-l'Étoile, France) matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) systems identified both colonies as *E. coli.* The 16 s rRNA sequencing concluded both isolates were *E. coli.* As automated systems in an ambient air were unable to grow capnophilic SCVs, antimicrobial susceptibility testing profile was determined through disk diffusion method [4]. With the exception of levofloxacin resistance, bacteria was susceptible to all other antimicrobials. From these findings, we concluded that this isolate was CO_2-dependent and had the ability to revert to its natural large form in the presence of CO_2.

Whole genome sequencing analysis by the MiSeq® system (Illumina, San Diego, USA) was performed to inspect assumed genes that contained previously-reported causative mutations for the *E. coli* SCV phenotype (*hemB*, *menC*, and *lipA* gene) [1, 5], but no genetic mutational variations were observed between the two strains. The *yadF* gene was not present in either strain, which is consistent with previous reports about capnophilic *E. coli* strains [6].

Discussion

The first *E. coli* SCV was reported in 1931, but there have been only few reports from clinical specimens [7–9]. Interestingly, this SCV strain was also capnophilic. The bacterial growth for reported capnophilic *E. coli* strains formed either large colonies in the presence of CO_2 or no colonies in the absence of CO_2 [2, 3]. To the best of our knowledge, this is the first report of *E. coli* with coexpressed SCV and capnophilic phenotype. Fortunately, this strain was susceptible to all other antimicrobials with the exception of levofloxacin, and therefore did not cause any severe outcome clinically. However, if this strain was to acquire drug resistance in the future, it is diagnostically crucial not

to misidentify or neglect such strain for proper therapeutic purposes.

Additional criteria including CO_2 conditions are needed because CLSI guidelines defining incubation conditions for *Enterobacteriaceae* involve 35 °C ambient air [4], which are unsuitable for growing capnophilic SCVs. We advise that all urine cultures should be incubated in an environment containing 5% CO_2 to avoid overlooking of such strains. Proper identification using alternative methods such as MALDI-TOF MS systems are necessary for these capnophilic strains.

Abbreviations
BAP: Blood agar plate; MALDI-TOF MS: Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; SCV: Small colony variants

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Availability of data and materials
Not applicable to this article as no datasets were generated or analyzed.

Authors' contributions
The study was planned and designed by YP, NP, NAP, MK, RD, JB, HS, and DY. MK collected the samples. YP, JB, HS, and DY conducted the experiments. The interpretation of the genetic results was done by NP, NAP, and RD. The manuscript was prepared by YP and JB. All authors contributed to and commented on the manuscript. All authors read and approved the final manuscript.

Ethics approval and conesnt to participate
The study was approved by the Institutional Review Board of Yonsei University Severance Hospital, Seoul, Korea (#2018–1951-001).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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