Manuscript title:

Isolation of an X-Factor Dependent but Porphyrin Positive *Escherichia coli* from Urine of a Patient with Hemorrhagic Cystitis

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Abstract :

An *Escherichia coli* isolate was recovered from a 92-year-old female patient with urinary tract infection. Gram-stained preparation of the urine sediment manifested some gram-negative rod-shaped cells, and the urine specimen culture yielded non-hemolytic colonies on sheep blood agar plate. However, no visible colonies appeared on modified Drigalski agar plate. The isolate was finally identified as an X-factor dependent *E. coli*. The interesting finding was that the isolate revealed positive reaction for porphyrin test despite the requirement of hemin. This finding suggested that some pyrrol-ring-containing porphyrin-compounds or fluorescent-porphyrins had been produced as chemical intermediates in the synthetic pathway from δ -amino-levulinic acid(ALA), although the isolate should be devoid of synthesizing hems from ALA. This was the first clinical isolation of such strain, indicating that the *E. coli* isolate should possess incomplete synthetic pathways of hems from ALA.

Introduction :

In routine clinical Microbiology laboratories, common pathogenic microorganisms for urinary tract infections are a mixture of aerobic or facultatively anaerobic gram-positive and gram-negative bacterial species [1]. Thus, a combination of 5% sheep blood agar and modified Drigalski or MacConkey agar are usually considered sufficient for the recovery of the microorganisms, although only in rare instances, when a more fastidious microorganism, such as *Neisseria gonorrhoeae* is suspected, an enriched medium, such as chocolate agar, should be necessary to determine the cause of infection.

We encountered an isolate from a urine specimen being capable of growing only on blood agar plates and being unable to grow on agar media without containing blood, including modified Drigalski agar medium. Moreover, the automated microbiological instrument, MicroScan WalkAway system (Siemens Healthcare Diagnostics, Tokyo, Japan) could not only correctly identify the isolate, but also failed to determined antimicrobial susceptibilities of the isolate.

Case report :

The isolate came from a 92-year-old female patient with mild sign of dementia. On her

admission to the outpatient Hana Medical Clinic, Shizuoka, Japan, on 21 Nov., 2009, she was complaining of pain in her left lower abdomen but was afebrile. Her urinalysis results demonstrated apparent hematuria with some gram-negative rod-shaped bacterial cells within abundant phagocytes. Under the clinical diagnosis of hemorrhagic cystitis, midstream urine was submitted to SRL Numazu Laboratory, Shizuoka, Japan for bacteriological examination. The causative gram-negative rod-shaped organism grew well on blood agar (Nippon Becton Dickinson Co., Ltd., Tokyo, Japan.) plate and was designated as 1121-09. The interesting finding was that no visible growth was observed on modified Drigalski agar (Nippon Becton Dickinson Co., Ltd., Tokyo, Japan) plate. Furthermore, the isolate failed to grow not only on Drigalski agar but also on DHL-, SS-, McConkey-, Heart-Infusion-, and Brain-Heart-Infusion agar (Eiken Chemical Co., Tokyo, Japan.) plates. For further microbiological examinations, the isolate grown on Blood agar plate was applied to MicroScan WalkAway system (Siemens Healthcare Diagnostics). The Neg Combo 6.11J panel in the system yielded the profile number 40004000 indicating unthinkable species names *i.e.*, *Shigella* sp., with identification probability of 94.25%. Indeed, *Shigella* species have hardly been reported as causative agents of urinary tract infections. In addition, the system displayed strange antimicrobial susceptibility results *i.e.*, “insufficient growth” and “indeterminable”. At this point, we could not grasp the reasons why the isolate failed to grow on many media and gave a feeble growth in the panel.

Discussion :

We previously reported the first successful isolation of *Dysgonomonas mossii* from intestinal juice of a patient with pancreatic cancer [2]. In that case, *D. mossii* isolate, in an analogous fashion, failed to grow on modified Drigalski agar plate, but yielded good growth on both sheep blood agar and chocolate agar (Nippon Becton Dickinson Co., Ltd., Tokyo, Japan) plates. After all, *D. mossii* isolate was found to be characteristic of requiring X-factor for growth, and demonstrated to be negative for porphyrin test [3].

Noticing our previous success in isolating *D. mossii*, we examined the X-factor requirement of the isolate 1121-09 and found that the isolate caused the satellite phenomenon around the X-factor-containing disk (Eiken Chemical Co., Tokyo, Japan.) on the Muller-Hinton (Eiken Chemical Co., Tokyo, Japan.) agar as shown in Figure 1, but failed to grow around V-disks,

clearly indicating the hemin dependence. However, the interesting finding was that the isolate demonstrated positive for porphyrin test. We therefore prepared the suspension of the isolate 1121-09 in the prompt broth in the MicroScan WalkAway system by adding hemin at the final concentration of 10 μ g/ml, and then the suspension was applied to the system using WalkAway NegCombo 6.11J panel (Siemens Healthcare Diagnostics). As a result, the system gave the profile number 53014012 demostrating *Escherichia coli* with excellent identification probability (>99.99%), and displayed that the isolate was highly susceptible to all the antimicrobials pre-arranged in the 6.11J panel. At the same time, we investigate the reliability of the 6.11J panel in the system by the addition of hemin in the bacterial suspension prompt media, and found that no effect was observed when the *E. coli* ATCC259222 strain was applied, yielding completely the same profile number 53115010 indicating *E. coli* with excellent identification probability (>99.99%) in both cases using the prompt with and without addition of hemin. In order to ensure the identification of the isolate 1121-09, the 16S rRNA gene was directly sequenced as described previously [4] usinsg a Taq DyeDeoxy Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA) and a model 3100 DNA sequencer instrument (Applied Biosystems, Foster City, CA). The sequence was retrieved from the Ribosomal Database Project databases [5]. Comparative sequence analysis showed 99% 16S rRNA sequence similarity to that of the type strain of *E. coli* ATCC25922 (DDBJ / EMBL / GenBank accession no. X80724). In consequence, the isolate 1121-09 was identified as *E. coli* with 99% identity, thus confirming the identification result of *E. coli* provided by the 6.11J panel of the system. In addition, the subsequent investigation revealed that API system (Sysmex bioMérieux Co., Ltd., Tokyo, Japan) and the ID-Test EB20 system (Nissui Seiyaku Co., Ltd., Tokyo, Japan), both having been widely adopted in clinical microbiology laboratories, correctly identified as *E. coli* with high probabilities (API-20E; profile number of 5144152 with 97.7%ID, and ID-Test EB20; profile number of 0110433 with 99.9%ID, respectively). These findings suggested that the broth media affiliated with respective system might contain at least a trace of hemin compounds.

In 1998, relatively similar hemin-requiring *E. coli* strain was isolated [6], and the reason for the hemin-dependence was due to lacking in heme biosynthesis genes (deletion of *hemB*). The gene *hemB* should be responsible for synthesizing porphobirinogen from δ

-aminolevulinic acid. The porphyrin test is known to react with pyrol-ring structured compounds including porphobilinogen. Theoretically, the deletion of *hemB* gene should bring about the negative reaction for porphyrin test, although not described in the paper [6]. Our isolate representing positive reaction for porphyrin test clearly demonstrated that the strain 1121-09 should possess metabolic pathway from δ-amino-levulinic acid to porphyrins, despite the inability to synthesize hems, implying the gene defects subsequent of *hemB* gene. This was, to the best of our knowledge, the first case report of isolating X-factor requiring clinical *E. coli* notwithstanding the positive reactions for porphyrin test. This phenomenon, which the isolate exhibited, was totally reminiscent of human porphyria [7].

In routine clinical microbiology, microbiologists sometimes encounter the isolates with unusual properties. Among them, the strains, which failed to grow on usual media, were apt to be missed or overlooked. We strongly considered that our valuable experience should be shared with many clinical microbiologists.

Although we fortunately isolated the strain 1121-09 since the blood agar was used in this case of urine material, in cases of fecal samples of suspected diarrheagenic *E. coli*, we could never isolate such strains since the blood or chocolate agar plates were not included in the usual set of isolation media. **For the future, it should be advisable to consider the possibilities that such diarrheogenic *E. coli* might be isolated from fecal specimens.** Implication of this type of X-factor requiring *E. coli* among clinical materials is an important finding, and our rare case report indicated that clinical microbiologist should pay well-suited attention to the isolation of this type of bacteria. Our case report is a noteworthy and useful piece of information in the field of clinical microbiology.

Conflict of interest.

The authors have declared that no conflict of interest exists.

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Figure legend.

Figure 1.
The growth of the strain-1121-09 apparently surrounded the X-factor impregnated strip on the Muller-Hinton agar, indicating that the isolate was hemin dependent.

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