



Small colony variant of *Escherichia coli* isolated from a recurrent urinary tract infection- a case report

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Abstract

Introduction: Small colony variant of *Escherichia coli* may cause persistent and recurrent infections in human. These slow growing phenotypic variants are difficult to identify and mislead the diagnosis which can lead to choice of inappropriate antibiotics. In this case report we are presenting a case of recurrent urinary tract infection which was associated with small colony variants of *E. coli*.

Case presentation: A two year old male child was admitted to the hospital with a complaint of passing thin stream of urine. One month earlier the child had been previously diagnosed with bilateral grade five Vesicoureteral reflux. Child had developed recurrent urinary tract infection caused by small colony variants of *E. coli*.

Conclusion: child had undergone bilateral ureter reimplantation as part of treatment to Vesicoureteral reflux. Since vesicoureteral reflex condition is one of the risk factor to develop urinary tract infections. Repeated exposure to antibiotic might have been induced stress condition to bacteria and emerged as slow growing sub population of *E. coli*.

Keywords: *Escherichia coli*, Small Colony Variant, Urinary Tract Infections.

1. Introduction

Urinary tract infections (UTI) are a common form of infection in infants and children. Diagnosis of UTI in young children and infants is very important as early detection plays a critical role for the preservation of renal function of the growing kidney (Habib S, 2012). Microbiological investigation of urine specimen is necessary for the identification of pathogens and antibiotic susceptibility (Hooton TM & Stamm WE, 1997). The most common pathogen associated with UTI is the uropathogenic *Escherichia coli* (UPEC), however some individuals have an increased predisposition to recurrences of UTI due to the initially infecting strain which even after elimination from urinary tract may persist in the faecal flora. Few studies reported that *Escherichia coli* can invade bladder epithelial cells and can form large bacterial inclusions after replication intracellularly (Foxman B, 1990 & Mulvey MA et al., 1998). Small colony variants (SCVs) are known as slow growing subpopulations that form small, non-pigmented and non-hemolytic colonies. Deficiencies in electron transport activities lead to auxotrophism for thymidine, menadione, or hemin causing the typical biochemical characteristics of the variants (Proctor RA et al., 2006 & L J Mandel et al., 1984). Studies show that 3-5 % of persistent infections may be caused by these small colony Variant *Escherichia coli* (Charlotte A. C. et al., 1946 & Roggenkamp A et al., 1998). Clinically these small colony variants are better able to persist in mammalian cells and are less susceptible to antibiotics (Harald S et al., 2003 & Proctor RA et al., 1995). Due to their unusual phenotypical and biochemical characteristics, they pose a challenge for clinical microbiologists. This problem might lead to diagnostic underestimation, which will cause therapeutic failures

in the clinical settings (Vaudaux P et al., 2006). Small colony variant *Escherichia coli* are reported to cause persistent and recurrent infections with more chances of treatment failure than those caused by wild type. However, there is limited literature regarding this. Here, we describe the case of a recurrent UTI infections caused by Small colony variant *Escherichia coli*, which occurred after undergoing surgery for bilateral grade five vesicouteral refluxes.

2. Case presentation

A two year old male child was admitted to the hospital with a complaint of passing thin stream of urine. One month earlier the child had been previously diagnosed with bilateral grade five Vesicoureteral reflux for which he had been surgically treated by bilateral ureter reimplantation. Following this, a complaint of UTI was reported and *Escherichia coli* was isolated. The patient on current admission had meatal stenosis with a complaint of poor stream of urine. The child was immunized as per the Indian primary immunization schedule. On Physical examination the child had a pulse rate of 90 per minute and Blood pressure 110/80 mmHg. Hematological findings showed 10% Monocyte, 1% Basophils, 51% Neutrophils, 38% Lymphocytes. Hemoglobin was 10.8 mg/dl, Platelet count was 465×10^3 per microliter and Total WBCs was found to be 20.1×10^3 per microliter. Urine specimen was collected after receiving consent from parents. Specimen was sent to laboratory for further investigations. Biochemical analysis of urine revealed that the pH was 6.5 with the color being pale yellow and specific gravity being <1.005. Traces of protein were present with sugar being absent in the urine. Blood urine was +1 (25 erythro-

cyte/microliter) and urine leukocyte was +3 (≥ 500 leukocyte/ml). Mid-stream urine was sent to microbiology laboratory and processed as per standard operating procedure. Macroscopic appearance of urine was turbid. Direct microscopy of urine specimen stained with gram stain showed numerous pus cells with gram negative bacilli. Semi-quantitative culture on blood agar yielded small colonies with colony count of $>10^5$ CFU/ml after incubated at 37°C for 24 hours. MacConkey agar plate showed pinpoint lactose fermenting colonies. On further incubation at 37°C for 72 hours the size of did not change (Figure 1 and 2). Isolated colonies were picked up for identification and phenotypic characterization. Motility was confirmed with hanging drop method, isolates were non-motile. It was Gram negative bacilli. Delayed Catalase reaction and oxidase reaction was negative. Glucose was fermented with acid and gas. Indole, Methyl red and nitrate reduction test was positive. Sugars like mannitol, lactose and xylose were fermented. It failed to ferment sucrose and sorbitol. Lysine was not decarboxylated. Colonies were suspected to be Small colony variant *Escherichia coli*. Further identification was confirmed by using automated identification system (Vitek-2 compact, Biomerieux, France). The result obtained was *Escherichia coli* with the similarity of 99%. Antimicrobial Susceptibility testing was performed by Kirby - Bauer disk diffusion method according to CLSI guidelines and the isolate was identified as Small colony variant *Escherichia coli* that did not grow on Mueller Hinton agar, so it was tested on Mueller Hinton agar supplemented with 5% human blood. It was resistant to Cefuroxime (30µg), Norfloxacin (10µg), Ampicillin (10 µg), Ceftriaxone (30 µg), Nitilmicin (30 µg), Amoxicillin - Calvulinic acid (20-10 µg), amikacin (30 µg), Aztreonam (30 µg), Gentamicin (10 µg), Cefepime (30 µg) and sensitive to Imipenem (10 µg), Cotrimoxazole (1.25-23.75 µg), Piperacillin - Tazobactam (100-10 µg) and Cefoperazone - Sulbactam (75-30 µg). This isolate was identified as ESBL producer. Repeated urine culture and sensitivity yielded the same results.

3. Discussion

To our knowledge, this is a first report of recurrent urinary tract infection caused by small colony variant of *Escherichia coli* with ESBL producer after surgical treatment of bilateral grade five vesicouteralreflex. There are only few case reports of small colony variant of *Escherichia coli* in the literature. The repeated admission of the patient with the laboratory results indicates that the present case was that of recurrent urinary tract infection. The infection caused earlier was by *Escherichia coli* sensitive to the aminoglycosides and beta-lactam antibiotics that are used in treatment of urinary tract infections. However the bacteria isolated later in the subsequent admission was not the same as the previous isolate. It turned out to be ESBL producer and also showed a change in the colony characteristics and biochemical tests results showed catalase weak positive and failed to ferment sorbitol. The bacteria did not grow in the routine culture media in 24 hours and took a longer time to grow at 37° C. In this case, the patient was treated with carbapenems during previous episode of UTI, unfortunately patient did not show recovery from the infection and currently progressed to recurrent UTI with *Escherichia coli* small colony variants, and probably indicating that due to the prolonged administration of the antibiotics given earlier due to surgery may have resulted in emergence of antibiotic stress induced small colony variants. In case of samples sent from patients who received antibiotic treatment for a long period, clinical microbiology laboratories should be on alert of small colony variant, and proper methods should be used to identify these pathogens. When there is an infection resistant to treatment, persistent or not responding to proper antimicrobial therapy small colony variant of *Escherichia coli* should be considered. In these situations, clinicians should ask the clinical microbiology laboratory to carefully look into identification of small colony variants, which might have an impact on the selection of treatment regime as small colony variants tend to resist intracellular killing and are more resistant to antibiotics.

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