The Ability of BBL™ CHROMagar™ Orientation to Recover Corynebacterium urealyticum

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REVISED ABSTRACT

BBL™ CHROMagar™ Orientation is a nonselective medium intended to isolate, differentiate and enumerate urinary tract pathogens. Corynebacterium urealyticum has been implicated as a causative agent of urinary tract infections. The purpose of this study was to determine if C. urealyticum could be recovered on CHROMagar Orientation. C. urealyticum is a strict aerobe, which requires incubation beyond 24 hours for recovery. The current recommendation for recovery of C. urealyticum from urine in suspected patients (those with symptoms, alkaline urine, or struvite crystals present) is to incubate a blood agar plate for 48 to 72 hours. In this study, 20 strains of C. urealyticum were evaluated for recovery on CHROMagar Orientation and Trypticase™ Soy Agar with 5% Sheep Blood (TSA II). Both media were inoculated with $10^3$ CFU per plate and incubated at 35°C in air and CO₂. Plates were examined for growth at 48 and 72 hours. Following 48 hours incubation, 17/20 (85%) strains were recovered on CHROMagar Orientation incubated in air, 4/20 (20%) strains were recovered on CHROMagar Orientation incubated in CO₂, and 20/20 (100%) strains were recovered on TSA II. After 72 hours incubation, all strains were recovered on CHROMagar Orientation incubated in air and 10/20 (50%) strains were recovered on CHROMagar Orientation incubated in CO₂. Strains recovered on CHROMagar Orientation when incubated in air demonstrated quantities of growth comparable to the TSA II control. C. urealyticum produced pinpoint colorless or white colonies on CHROMagar Orientation. On TSA II, colonies were small, non-hemolytic and white to grayish in color. This study suggests that BBL CHROMagar Orientation can be used to recover Corynebacterium urealyticum when incubated in air and held 48 to 72 hours.

INTRODUCTION

Corynebacterium urealyticum plays a role in urinary tract infections in patients who are immunocompromised, elderly or who have been urologically manipulated. It may cause acute or chronic infections of the lower urinary tract if there is underlying renal or bladder disease and may also cause urinary tract infections like pyelonephritis or polyuretheritis. C. urealyticum is primarily associated with alkali-encrusted cystitis. C. urealyticum is urealytic, which causes ammonia to form, producing an alkaline urinary pH. The urine becomes saturated with ammonium magnesium phosphate (struvite) and calcium phosphate that precipitate, causing the encrustation.

The isolation and identification of Corynebacterium urealyticum is particularly important in renal transplant patients with post-transplant UTI. Alkaline urine pH, struvite crystals, leukocytes and erythrocytes are frequent findings on urinalysis. If urinalysis is suggestive or a patient has clinical evidence of bacteriuria with a negative standard urine culture, physicians should consider this pathogen and request the laboratory to specifically culture for C. urealyticum. Recommended laboratory procedures include extending incubation time for blood or CLED agar to 48 to 72 hours to improve recovery and investigating any diphtheroid bacilli isolated from the urine culture. C. urealyticum is a strict aerobe and incubation in air is recommended.

BBL CHROMagar Orientation is a nonselective medium for the isolation, differentiation and enumeration of urinary tract pathogens. CHROMagar Orientation is composed of a nutritive base and a chromogenic mix of artificial substrates to aid in the identification of urinary tract pathogens. In this study, CHROMagar Orientation was evaluated for its ability to recover Corynebacterium urealyticum.
MATERIALS AND METHODS

Materials

- BBL™ CHROMagar™ Orientation (Cat. Nos. 254102 and 215081)
- Trypticase™ Soy Agar with 5% Sheep Blood (TSA II) (Cat. No 221261)
- BBL™ Nitrate Broth (Cat. No 221830)
- BBL™ Urea Agar (Cat. No. 221096)
- BBL™ CTA Medium with Dextrose (Cat. No. 221633)
- BBL™ CTA Medium with Maltose (Cat. no. 221637)

Test Strains

- *Corynebacterium urealyticum*: 3 ATCC™, 21 industrial/clinical

Method

Twenty-one (21) strains of suspected *C. urealyticum* were tested using tube biochemicals to aid in speciation. Three ATCC strains were tested in parallel. Gram stain, catalase, urea, nitrate, dextrose and maltose were performed on each strain. The following chart outlines the expected results of biochemical tests indicative of *C. urealyticum*.

The picture represents expected biochemical tube reactions for *Corynebacterium urealyticum* strains. Urea, on left, is positive, followed left to right by a negative nitrate, negative glucose and negative maltose. Seventeen of the 21 suspected strains were identified as *C. urealyticum*.

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Each ATCC strain (N=3) and the test strains, biochemically identified as *Corynebacterium urealyticum* (N=17) were inoculated to CHROMagar Orientation and TSA II plates. Inocula were prepared in TSB and diluted in sterile DI water from fresh overnight cultures and streaked in four quadrants for isolation. All *Corynebacterium urealyticum* strains were inoculated at a concentration of $10^3$ CFU per plate. Media were incubated at 35°C in air and CO₂ and examined at 24, 48 and 72 hours. At 24 hours, growth was not adequate to interpret. Growth on CHROMagar Orientation was compared to TSA II (a nonselective control) for the recovery of *C. urealyticum* at 48 and 72 hours. Colonies of *C. urealyticum* are pinpoint colonies that are colorless to white after 48 to 72 hours incubation on CHROMagar Orientation.
RESULTS

Detection of *Corynebacterium urealyticum* on CHROMagar Orientation and TSA II at 24 hours required extended incubation. At 48 hours incubation, all confirmed test strains (20 of 20) were detected on TSA II as small colonies. On CHROMagar Orientation incubated in air, 17 of 20 strains, (85%), were detected as pinpoint colonies. Four strains (20%) were recovered from CHROMagar Orientation incubated in CO₂ after 48 hours. Following extended incubation to 72 hours, recovery on TSA II and CHROMagar Orientation incubated in air was 100%. Only 10 of 20 strains were recovered on CHROMagar Orientation incubated in CO₂ after 72 hours.

![Bar chart showing % Recovery of C. urealyticum](chart.png)

DISCUSSION

Urinalysis frequently provides information suggestive of infection with *Corynebacterium urealyticum*, such as an alkaline pH and the presence of WBCs, RBCs and struvite crystals. If a physician suspects *Corynebacterium urealyticum* as a potential cause of infection, this information should be communicated to the clinical microbiology laboratory. The laboratory should take steps to improve the chance for recovery of this organism by extending the incubation time to 48–72 hours. This study shows that recovery of *Corynebacterium urealyticum* on CHROMagar Orientation is possible when incubation time is extended to 48–72 hours and when incubation is done in air.

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- CHROMagar is a registered trademark of Dr. A. Rambach.
- ATCC is a registered trademark of the American Type Culture Collection.
CONCLUSIONS

- In laboratories currently using CHROMagar Orientation as a primary plating medium for urine cultures, extended incubation to 48–72 hours improves percent recovery of *C. urealyticum*.

- Incubation of CHROMagar Orientation plates in air improves recovery of *Corynebacterium urealyticum* strains.

- BBL CHROMagar Orientation supports recovery of *C. urealyticum*.