

Challenge M134-2

February 2014

Midstream urine: $>100 \times 10^6$ cfu/L *Corynebacterium urealyticum*

HISTORY

The challenge was sent to category A and B laboratories. The sample simulated midstream urine obtained from an 88 year old male patient with urinary tract infection (UTI) symptoms.

Laboratories were expected to isolate, identify and report *C. urealyticum* as well as report the susceptibility results to penicillin, gentamicin, and vancomycin.

This sendout constituted the first *C. urealyticum* challenge sent by CMPT.

CMPT QC

CMPT quality control yielded $>100 \times 10^6$ cfu/L of *Corynebacterium urealyticum* viable for 19 days.

SURVEY RESULTS

Reference Laboratories:

Three reference laboratories did not obtain growth and thus did not perform colony count, identification, or susceptibility testing.

Colony count: 12/15 (80%) laboratories reported $\geq 10-100 \times 10^6$ cfu/L,

Identification: 12/15 (80%) laboratories reported *C. urealyticum*,

Antimicrobial susceptibility testing: 10 laboratories reported the strain resistant to penicillin (10/12 with growth: 83%), 2 did not report pen-

Table 1. Results reported - Colony count

Colony count x 10^6 cfu/L	Total	Grade
$\geq 100, \geq 10$	66	4
10-100	2	4
$>100,000$ cfu/L	1	1
no growth, negative*	10	0
no report	2	0
snp	3	ungraded
Total	74	

*2 labs reported using Chromagar and one reported using dipslide.

MAIN EDUCATIONAL POINT from M134-2

1. *Corynebacterium urealyticum* has been strongly associated with urinary tract infections and should be identified and reported when found to be pure or predominant growth in significant amounts.

icillin susceptibility; 8 laboratories reported the isolate susceptible to gentamicin (8/12 with growth: 67%) while 1 laboratory reported it resistant; 2 laboratories indicated they would refer and 1 laboratory did not report gentamicin susceptibility; 11 laboratories reported the isolate susceptible to vancomycin (11/12 with growth: 92%) and 1 laboratory did not report.

Consensus was obtained for the following components: colony count, identification, and susceptibility to penicillin and vancomycin. These components were graded.

No consensus was reached for gentamicin susceptibility therefore, this component was not graded.

Participants

Colony count (Table 1): 68/74 (92%) participants correctly reported a colony count of $10 - >100 \times 10^6$ cfu/L to receive a grade of 4. One laboratory reported the colony count in non-standard units and thus, was graded 1. Those laboratories that did not obtain growth (10) were graded 0. Of these laboratories, three indicated they had plated the sample on Chromagar or used dipslides; seven laboratories did not indicate the medium used.

Identification (Table 2):

41/74 (55%) participants correctly reported the isolate as *C. urealyticum* and were graded 4. 7 more laboratories reported the isolate as *Corynebacterium* species, these laboratories were graded 3, or 4 if there was an intention to refer for further identification.

The remaining laboratories reported the isolate as gram positive bacilli, coccobacilli, diphtheroids, gram positive cocci or misidentified it as *Gemella haemolysans* (1), *Kocuria* species (6), *Micrococcus* species (4) or coagulase negative

Grading

Maximum grade: 16

Reporting $\geq 10-100 \times 10^6$ cfu/L was graded 4.

Not using standard units in colony count was graded 1.

Not obtaining growth was graded 0.

Reporting *Corynebacterium urealyticum* was graded 4.

Reporting *Corynebacterium* species and refer for further identification was graded 4.

Reporting *Corynebacterium* species without referring was downgraded to 3.

Reporting gram positive bacilli was graded 1.

Reporting diphtheroids, gram positive cocci or the wrong identification was graded 0.

Reporting penicillin resistant was graded 4.

Reporting vancomycin susceptible was graded 4.

Not reporting results for the antibiotics graded was graded 0 for each agent not tested.

Not reporting results is always graded 0.

staphylococcus (2). For grading please refer to table 2.

Susceptibility (Tables 3A – C): The 30 participants that reported results for penicillin, reported the isolate resistant and were graded 4 (table 3A). Six laboratories that did not report susceptibility to penicillin were graded 0; those laboratories that referred for testing were ungraded.

No consensus was reached for gentamicin susceptibility results; table 3B is included for information purposes only.

31 participants reported susceptibility results to vancomycin, all of them reported the isolate as susceptible and received a grade of 4 (table 3C). Laboratories that neither reported vancomycin results nor referred the isolate for testing were graded 0.

COMMENTS ON RESULTS

Laboratories that were unable to isolate the intended organism should consider their media selection. *Corynebacterium* species, including *C.urealyticum*, grow readily on 5% blood agar.

Several misidentifications were submitted. All of the incorrect organism identifications were

species of gram positive cocci. These laboratories are encouraged to include a direct colony Gram stain whenever the Gram classification of an organism is not apparent.

ISOLATION and IDENTIFICATION

The Gram staining morphology of the cells is very important for the correct identification of the genus. *Corynebacterium urealyticum* is a gram positive bacillus with non-parallel, curved sides frequently arranged in groups ¹.

Morphology, size, pigment, odor, and hemolysis of colonies are also valuable criteria in the differential diagnosis of coryneform bacteria ².

All medically relevant *Corynebacterium* species are catalase positive and non-motile. A prolonged incubation is usually needed ³ and thus, *C. urealyticum* can be easily missed at 24h. Colonies are round, smooth and whitish, between 2 and 4mm after 48 to 72hs incubation at 37C on 5%sheep blood agar ^{1,3}.

C. urealyticum is a strict aerobe and has very strong urease activity giving the urease test is positive in a few minutes ². This strong urealytic capacity of *C. urealyticum*, leads to ammonia formation, and thus, alkalinisation of the urine

Table 2. Identification results

Reported results	A Labs	B Labs	Total	Grade
<i>Corynebacterium urealyticum</i> ± presumptive ± refer	37	3	40	4
gram positive bacilli, suggestive of <i>Corynebacterium urealyticum</i> , refer	1		1	4
<i>Corynebacterium</i> species, refer ± presumptive	5		5	4
<i>Corynebacterium</i> species	2		2	3
gram positive coccobacilli, possible corynebacteria, refer	2		2	3
gram positive bacilli		1	1	1
diphtheroids		1	1	0
gram positive cocci , refer	4		4	0
coagulase-negative Staphylococcus	2		2	0
<i>Gemella haemolysans</i>	1		1	0
<i>Kocuria</i> species (<i>Kocuria</i> species - 1, <i>K. rosea</i> , refer -2, <i>K. varians</i> , refer -3)	4	2	6	0
<i>Micrococcus</i> species, ± refer	2	2	4	0
unable to identify organism, refer**		1	1	0
no growth, no clinically significant growth	10		0	0
no report	1		1	0
Sample not normally processed	3		3	ungraded
Total	64	10	74	

** Laboratory did not report Gram morphology

Table 3. Susceptibility results reported

3A- Penicillin	Total	Grade
Resistant	30	4
no Penicillin report	6	0
no ID report, n/a	1	ungraded
no growth, n/a	10	ungraded
refer, snnp	37	ungraded
Total	84	
3B- Gentamicin	Total	Grade
Susceptible	23	ungraded
Resistant	1	ungraded
no Gentamicin report	5	ungraded
comment ***	1	ungraded
no growth, n/a	10	ungraded
no ID report, n/a	1	ungraded
refer, snnp	43	ungraded
Total	84	

***comment: not part of our laboratory procedure. Would be reviewed with microbiologist. If needed it would be forwarded to a reference laboratory.

3C- Vancomycin	Total	Grade
Susceptible	31	4
no vancomycin report	5	0
no growth, n/a	10	ungraded
no ID report, n/a	1	ungraded
refer, snnp	37	ungraded
Total	84	

with the consequent precipitation of struvite (NH₄MgPO₄) crystals ⁴.

Urine samples that have certain characteristics suggest the presence of *C. urealyticum*: pyuria-hematuria, alkaline pH, and the presence of struvite crystals, although these three signs are not always observed ¹.

C. urealyticum is correctly identified by commercial identification systems ².

ANTIMICROBIAL SUSCEPTIBILITY

Corynebacterium urealyticum is typically resistant to multiple antibiotics. It is normally highly resistant to β-lactams and aminoglycosides, but normally susceptible to vancomycin. Susceptibility to quinolones, erythromycin, rifampicin, and tetracycline varies ^{1,5}.

CLINICAL RELEVANCE

Corynebacterium urealyticum is a commensal skin organism ⁶ and has been associated with urinary tract infections.

Breakdown of urea by a strong urealytic organism like *C. urealyticum* leads to the formation of ammonium (NH₃) which increases pH favoring precipitation of NH₄⁺ with magnesium and phosphate leading to the formation of struvite ⁷.

These mineral compounds are responsible for encrustations along the infected mucosa and stone formation. These encrustations can lead to obstructive uropathy and other complications. ^{3,4}

Prolonged vesical and ureteral catheterization is the most important risk factor for developing encrusted cystitis (EC) and encrusted pyelitis (EP), the urothelial trauma created with these procedures increase the risk of simple urinary tract infections turning into EC or EP ⁶.

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