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DETECTING CLINICALLY RELEVANT MICROORGANISMS: WE CAN DO BETTER

Hypothesis / aims of study
To compare the standard urine culture to an improved urine culture protocol for detection of clinically relevant microorganisms.

Study design, materials and methods
We prospectively enrolled 115 urogynecologic patients and dichotomized the group based on their UTI perception, using a Y/N response to "Do you feel you have a UTI?" In addition to a standard clinical urine culture of the catheterized urine specimens, bacterial growth was assessed using 3 versions of an expanded quantitative urine culture (EQUC) protocol, which uses different media, an expansion of environmental culturing conditions, and 3 volumes of urine plated (1µL, 10µL and 100µL) instead of the 1µL used in standard culture practice (Table 1). Bacterial growth detected with EQUC was identified using Matrix Assisted Laser Desorption/Ionization - Time of Flight Mass Spectrometry (MALDI-TOF MS). Microbiota diversity was assessed using the average number of unique species per urine specimen. Women were treated clinically based on results from the clinical standard urine culture alone.

Results
The 100µL EQUC protocol detected significantly more unique species (82) compared to the standard culture (8). Use of the 10 µL and 1 µL EQUC protocols detected intermediate amounts of unique species (48 & 31, respectively). The standard culture missed 75% (93/124) of the uropathogens detected using the EQUC protocols. In the YES cohort, the standard culture missed 50% (26/52) of the uropathogens. Standard culture detected most *Escherichia coli* (92% - 22/24), but detected only a minority of all other uropathogens (9% - 9/100).

Many uropathogens were detected by EQUC at levels (colony forming units per milliliter – CFU/mL) below the accepted threshold for UTI diagnosis (≥10^5 CFU/mL). Uropathogens detected by EQUC in the YES cohort were at higher average CFU/mL than those detected in the NO cohort. Uropathogens detected in the YES cohort however, still generally fell below the accepted threshold and thus would not be considered to be causing infection despite the patient reporting UTI symptoms.

By surveying the detection of uropathogens across all EQUC plating conditions, an optimal EQUC version was determined. This optimal EQUC version [100µL of urine plated onto Blood (5% CO_2), CNA (5% CO_2), and MAC (O_2) with 48 hours of incubation] detected more uropathogens 78% (97/124) than standard culture 25% (31/124) (Fig. 1).

Interpretation of results
Improved methods of uropathogen detection may aid clinicians in selecting treatment for women reporting UTI symptoms. These data do not support the use of ≥10^5 CFU/mL as a threshold for UTI diagnosis, as the majority of women in the YES cohort, had uropathogens present at lower levels. The optimal form of EQUC, as determined by these data, results in a dramatic enhancement of uropathogen detection. Implementation of this new protocol is feasible for clinical microbiology laboratories, as it only requires an adjustment in incubation conditions, and the use of one additional plate. On the downside, this new protocol would require longer urine culture incubation times, which would delay treatment.

TABLE 1.

Protocol (Volume)	Media	Condition	Incubation Time
Standard (1µL)	Blood & MacConkey	Aerobic, 35° C	24 hr.
EQUC (1µL, 10µL, 100µL)	Blood, Chocolate, & CNA	5% CO_2, 35° C	24 hr. & 48 hr.
	Blood & MacConkey	Aerobic, 35° C	24 hr. & 48 hr.
	Anaerobic Blood	Anaerobic, 35° C	48 hr.
	Anaerobic Blood	Campy gas mixture (5% O_2, 10% CO_2, 85% N), 35° C	48 hr.

FIGURE 1. Uropathogen Detection by EQUC. Total number of uropathogens detected by each plating condition as assessed by the use of EQUC. Use of Blood (5% CO_2), CNA (5% CO_2), and MAC (O_2) incubated for 48 h at 35ºC, resulted in the most efficient and effective detection of uropathogens.

Uropathogen Detection		Blood CO_2	Chocolate CO_2	CNA CO_2	Anaerobic	Blood O_2	MacConkey O_2	Anaerobic CAMPY
Actinobaculum schaalii	5	2	0	4	3	0	0	4
Aerococcus urinae	11	5	5	7	4	4	0	0
Alloscardovia omnicolens	6	3	1	4	4	0	0	1
Candida albicans	2	2	2	2	2	2	0	2
Candida parapsilosis	4	1	1	2	2	0	0	1
Citrobacter koseri	1	1	1	0	1	1	1	0
Corynebacterium riegelii	3	1	1	0	2	1	0	1
Corynebacterium urealyticum	1	1	0	0	0	1	0	0
Enterobacter aerogenes	3	2	2	0	1	1	2	2
Enterococcus faecalis	12	5	4	7	5	7	0	6
Escherichia coli	24	22	22	3	23	22	23	17
Klebsiella pneumoniae	8	5	4	0	7	6	5	5
Proteus mirabilis	1	1	1	0	1	1	1	1
Pseudomonas aeruginosa	1	1	1	0	1	1	1	1
Serratia marcescens	1	0	1	0	1	0	0	0
Staphylococcus aureus	5	2	2	2	1	2	0	2
Streptococcus agalactiae	10	8	6	5	6	6	0	5
Streptococcus anginosus	26	13	8	19	13	2	0	13
TOTAL	124	75	62	55	77	57	33	61

Concluding message

The optimal form of EQUC markedly improves uropathogen detection. These findings support the necessity for an immediate change in urine culture procedures to improve patient care.

Disclosures

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