Correlating Culture Colony Forming Units to PCR Cycle Threshold Values in Urinary Tract Infections

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INTRODUCTION

Urinary tract infections (UTIs) are one of the most prevalent infections impacting more than 150 million people worldwide every year.[1] UTI hospitalizations had an estimated annual cost of $2.8 billion dollars in 2011.[2] This cost is compounded by the increasing threat of carbapenem resistant bacteria[3] and other antimicrobials. Given recent reports showing urine cultures report 'no growth' in approximately 90% of cultures submitted, emergent Nucleic Acid Amplification Tests (NAAT) targeting several pathogens responsible for UTI, may enable more rapid UTI diagnosis for better clinical outcomes and patient management decisions.[4]

This study was undertaken to compare the HealthTrackRx UTI syndromic multiplex PCR panel (HTRX UTI panel) with traditional culture to establish a direct correlation between cycle threshold (CT) values and colony forming units per ml (CFU/ml) of UTI causing pathogens. Here we describe the results of the application of the semi-quantitative thresholds to UTI PCR results obtained from specimens previously tested by traditional urine culture.

The clinical implications from this study include the addition of semi-quantitative values on UTI PCR results allowing comparison to traditional urine culture results and by so doing, provide an enhanced results report which will assist the provider with more actionable information and will help further guide more informed treatment decisions.

MATERIALS & METHODS

Patient specimens were obtained which were previously tested using traditional urine culture procedures. A total of 300 de-identified urine specimens were analyzed using the HealthTrackRx UTI PCR assay and CT values were obtained and compared to the previously reported urine culture results.[5]

To validate our PCR result we used the semi-quantitative thresholds determined from CT-CFU/ml studies to convert the CT results obtained after urine specimens were tested using the HealthTrackRx UTI PCR into CFU/ml values (Poster # ID018).

We then analyzed the data to determine concordance between the two values. The CT-CFU/ml studies were performed using three Gram negative (*E. coli*, *Klebsiella* and *Pseudomonas*), and two Gram positive bacteria (*S. aureus* and *Enterococcus*). Our evaluation of the semi-quantitative threshold primarily focused on these five pathogens. However, we then applied the semi-quantitative criteria to other pathogens with previously reported CT-CFU/ml values.

RESULTS

Gram Negatives

Dilution	CT Value Range (Average)	Report
> 10^5 CFU/ml	< 23	Detected >10^5 CFU/ml
< 10^5 CFU/ml	23 - 28	Detected <10^5 CFU/ml

Gram Positives

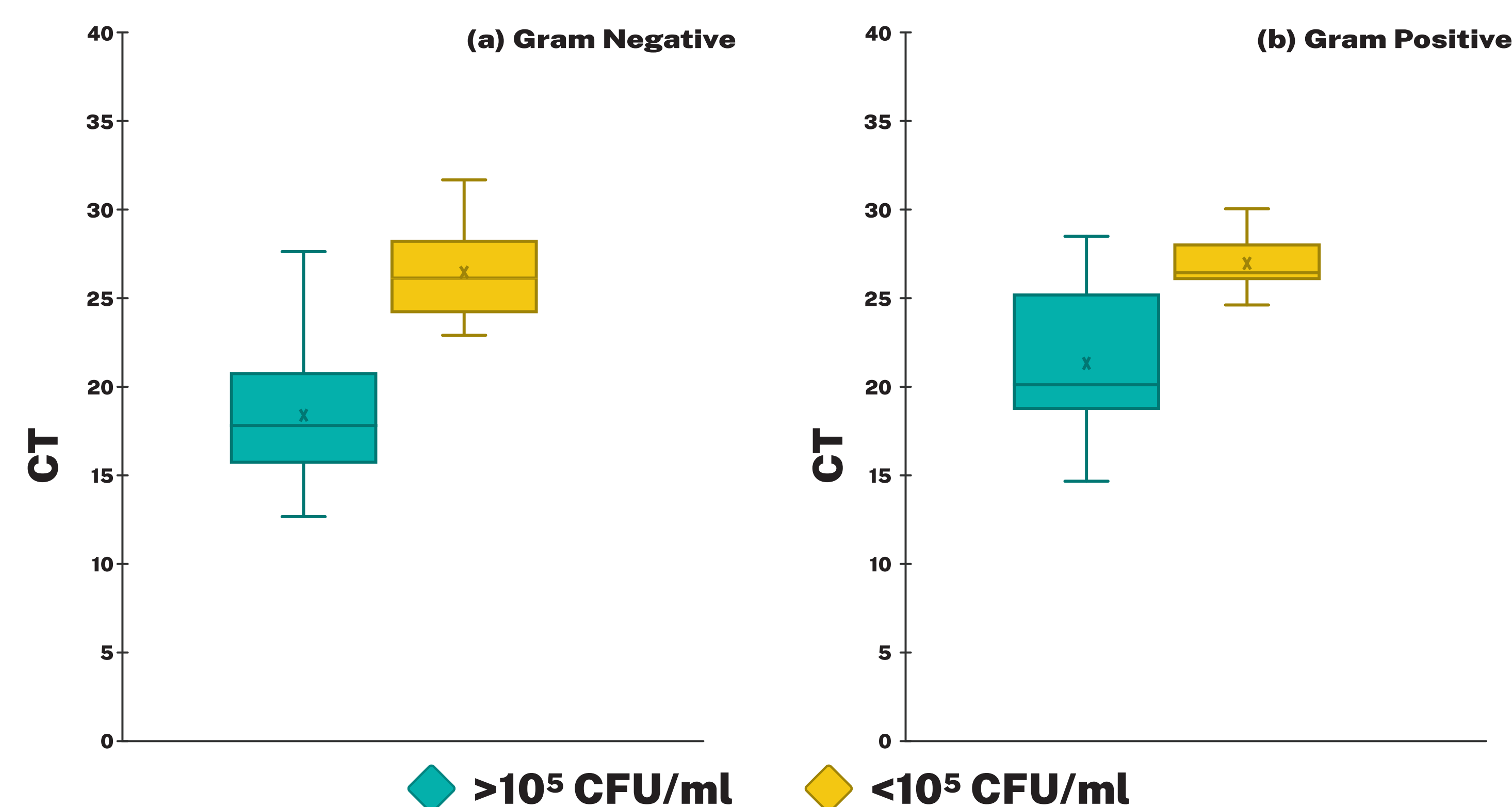| Dilution	CT Value Range (Average)	Report
> 10^5 CFU/ml	< 26	Detected >10^5 CFU/ml
< 10^5 CFU/ml	26 - 30	Detected <10^5 CFU/ml

Table 1: Semi-quantitative criteria for CT values.

Figure 1: **CT value distribution of Gram negative and positive bacteria detected at greater and less than 10^5 CFU/ml in urine sample cultures.** (a) Gram negative: >10^5 CFU/ml – 17.7 (15.63, 20.82); <10^5 CFU/ml – 25.92 (23.93, 28.09). (b) Gram positive: >10^5 CFU/ml – 21.06 (19.95, 24.28); <10^5 CFU/ml – 28 (26.33, 27.83).

	Reported CFU/ml Result	Count	Agreement	Non-Agreement	Overall Percent Agreement
Enterobacter spp.	>10^5	6	6	0	100
	<10^5	1	0	1	*
E. coli	>10^5	90	82	8	91.1
	<10^5	13	11	2	84.6
Klebsiella spp.	>10^5	11	9	2	81.8
	<10^5	5	5	0	100
Psuedomonas spp.	>10^5	3	2	1	66
	<10^5	0	0	0	*
S. marcesans	>10^5	1	1	0	100
	<10^5	0	0	0	*
Citrobacter spp.	>10^5	1	1	0	100
	<10^5	2	0	2	*
Proteus	>10^5	4	3	1	75
	<10^5	1	1	0	100
S. agalactiae	>10^5	1	1	0	100
	<10^5	4	0	4	*
S. aureus	>10^5	4	3	1	75
	<10^5	0	0	0	*
Enterococcus spp.	>10^5	4	4	0	100
	<10^5	5	4	1	80

Table 2: **Percent agreement between CFU/ml values and CT values after application of semi-quantitation criteria.** The table shows the percent agreement of CT values after application of semi-quantitative criteria to the reported CFU/ml value. The overall concordance between reported CFU/ml values and the CT values after application of semi-quantitative criteria was 89%.

Based on CT-CFU/ml studies performed at HealthTrackRx, a CFU/ml value of 10^5 for all Gram negative bacteria correlates to an average CT value of 23. The corresponding CT value for Gram positives is 26 for 10^5 CFU/ml. To validate the semi-quantitative threshold values, we applied them to data obtained from a comparison study of traditional urine culture to the UTI PCR assay (Poster# ID018). Our results show that out of 10^3 *E. coli* isolates detected by urine culture and by the UTI PCR assay, there was 91.1% agreement between isolates reported as greater than 10^5 CFU/ml in urine culture and isolates with a UTI PCR CT value of lower than 23.

For isolates reported as lower than 10^5 CFU/ml, there was agreement between 84.6% (11/13). The two isolates which were discordant had CT values of 18.69 and 22.96 and would have been reported as greater than 10^5 CFU/ml after application of our semi-quantitative criteria. Overall, there was 88.89% concordance between all *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *Enterococcus spp.* and *S. aureus* detected in this cohort and the reported CFU/ml values. Table 2 shows the results when the semi-quantitative criteria was applied to CT values of Gram positive and Gram negative bacteria reported in the study.

CONCLUSIONS

- The purpose of this study was to compare the HealthtrackRx syndromic PCR UTI PCR test to traditional urine culture. To this end we have sought to create a method to allow providers to be able to directly correlate the results from the HealthTrackRx syndromic PCR with the familiar traditional urine culture results.

- To measure the effectiveness of our semi-quantitative criteria we have applied them to results from previously analyzed urine specimens. Based on our studies a CFU/ml value of 105 for all Gram negative bacteria was shown to correlate to an average CT value of 23. The corresponding CT value for Gram positive bacteria, based on our studies, is 26 for 105 CFU/ml (*Table 1*). These values were then applied to the previously resulted cohort of urine specimens.

- The application of the semi-quantitative criteria to CT values obtained from previously tested urine culture specimens showed an overall agreement of 88.89% when applied to Gram negative and Gram positive isolates (*Table 2*). Only 15 isolates were non-concordant with their respective reported CFU/ml value.

- The outcome of this clinical validation study suggests the semi-quantitative criteria we have developed can be successfully applied to CT values from the UTI PCR assay. The concordance between CT values after application of the derived semi-quantitative criteria was approximately 90%. The inclusion of this additional information will further assist providers seeking to interpret PCR results and to make more informed treatment decisions.

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