



Prospective Implementation of a Point-of-Care PCR-Based Detection Method to Guide Antibiotic Use Prior to Prostate Biopsy Compared to Targeted Prophylaxis and Physician Choice

Michael A. Liss, Allison Sherrill, Shane Barney, Andrea Yunes, Evenki Sokurenko, and Brian Wickes

OBJECTIVE	To perform pilot testing regarding implementation of a point-of-care qPCR-based test (EST200) targeting bacterial clonal groups representing the majority of sepsis-causing <i>Escherichia coli</i> before prostate biopsy to determine antibiotic selection.
MATERIALS AND METHODS	After IRB approval, we obtained rectal swabs to compare real-time qPCR analysis on a Rotor-Gene Q instrument (Qiagen, Hilden, Germany) to standard culture on ciprofloxacin infused (10mg/L) MacConkey agar and susceptibility testing. Techniques are compared by an area under the receiver operative curve (AUC).
RESULTS	A total of 140 men participated in the study, 102 prebiopsy cultures were utilized to guide prophylaxis. We did not meet our accrual for the randomized portion of the clinical study, yet we did randomized 38 men without prebiopsy cultures to physician choice of antibiotic versus PCR-based approach. Regarding predicting Fluoroquinolone Resistant (FQR) at biopsy, prebiopsy cultures had an AUC of 0.91 (95%CI 0.84-1.00, $P > .001$) and polymerase chain reaction (PCR) had an AUC of 0.71 (95%CI 0.58-0.84, $P = .005$) (AUC comparison; $Z = 2.31$, $P = .02$). PCR correctly identified 4 of 5 FQR specimens. The PCR test attained an AUC of 0.79 (95%CI 0.56-1.00, $P = .044$) for detection of total FQR on the day of the biopsy. Risk-based techniques may over-compensate with additional antibiotics (21% versus 0%, $P = .10$).
CONCLUSION	EST200 is a rapid PCR-based microbial detection system that has moderate ability to detect total FQR at the time of biopsy. Our study is underpowered, yet provide opportunities to improve the point of care PCR method, such as table top testing in less than 20 minutes and include additional antibacterial resistant genes. UROLOGY 129: 87–91, 2019. © 2019 Elsevier Inc.

The American Urological Association Best Practice Policy Statement on Urologic Surgery Antimicrobial Prophylaxis has recommended ciprofloxacin 500 mg twice daily to start the morning of prostate biopsy lasting 2 doses (24 hours).¹ The strategy is successful; however, over time, the most common etiology of infection after a prostate biopsy is resistance to fluoroquinolones.²

The rise in infections has led urologists to investigate various prevention strategies to include additional prophylactic antibiotics.^{3,4} Another strategy for prophylaxis was to perform rectal culture for the presence of Fluoroquinolone Resistant (FQR) *E coli* 1-2 weeks prior to the prostate biopsy and, if present, use susceptibility results to choose an alternative antibiotic prophylaxis—this was termed “targeted prophylaxis.”⁵ Targeted prophylaxis allows for a significant reduction in the use of multiple antibiotics compared to augmented prophylaxis.

Two of the main arguments against targeted prophylaxis discussed by urologists at the AUA Quality Improvement Summit 2014 included, first, that the change in antibiotics was not recognized in the Surgical Quality Improvement Program (SCIP) measures and, second, obtaining a rectal culture before transrectal prostate

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From the University of Texas Health Science Center San Antonio San Antonio, TX; the South Texas Veterans Healthcare, TX; the University of Washington, WA; and the Department of Microbiology, University of Texas Health San Antonio, TX

Address correspondence to: Michael A. Liss M.D., M.A.S., F.A.C.S., Department of Urology, 7703 Floyd Curl Drive, San Antonio, TX 78229. E-mails: liss@uthscsa.edu; michael_liss88@yahoo.com

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biopsy (TPB) is logistically inconvenient.⁶ The bacteria causing infection and particular sepsis are in the rectum at the time of prostate biopsy. We reported ST131 *E coli* accounts for 70% of post-TPB sepsis.⁷ Therefore, we propose to continue the standard fluoroquinolone prophylaxis and then select a particular antibiotic for augmentation by utilizing a point-of-care (POC) test polymerase chain reaction (PCR)-based detection pilot study to understand barriers of implementation and potential reduce antibiotic overuse.

MATERIALS AND METHODS

Population: We prospectively enrolled men over the age of 30 presenting to the Audie Murphy Veterans hospital (AMVA) in San Antonio, Texas, for TPB in a clinical trial. We excluded men with allergies or other adverse reactions to quinolones, patients with previous prostate biopsy infection, and patients with hepatic or renal insufficiency in which a fluoroquinolone is contraindicated. We registered the trial on ClinicalTrials.gov (NCT03040050).

Protocol/Randomization: After IRB approval and the consenting process, we obtained rectal swabs in group 1 (usual care) after the decision to proceed to TPB. The rectal culture is the

standard of care at our institution. The swab has 2 swab culturettes. We send 1 culturette to our clinical microbiology lab and the second culturette to our genitourinary repository. From the repository, the EST200 test was performed to compare to the standard of care. Men in group 2 (intervention) either did not have a rectal culture completed or the culture was more than 30 days old. We then collected a new rectal swab on the day of prostate biopsy approximately 90-120 minutes before the procedure. Rectal swabs had 2 culturettes. Again, we took 1 culturette to the institutional microbiology lab for standard culture and the other for immediate POC PCR testing. Men enrolled at the AMVA before prostate biopsy then were randomized in 1:1 fashion using <https://www.randomizer.org/> to rapid multiplex qPCR or physician choice (Figure 1). All patients were prescribed the standard ciprofloxacin 500 mg twice daily to start the morning of prostate biopsy, lasting 2 doses. If randomized to the PCR approach, patients were instructed to arrive at least 1.5 hours before their scheduled procedure time and were still to take their ciprofloxacin; however, we did not adjust group 1 (usual care) patient's time. After consent and initial questionnaire, we performed a rectal swab with 2-swab culturette. The standard-of-care culturette was sent to microbiology for plating on MacConkey agar infused with 10 ug/mL of ciprofloxacin (Hardy Diagnostics, Santa Maria, CA) (study gold standard). We sent the second culturette for direct PCR analysis. In group 2

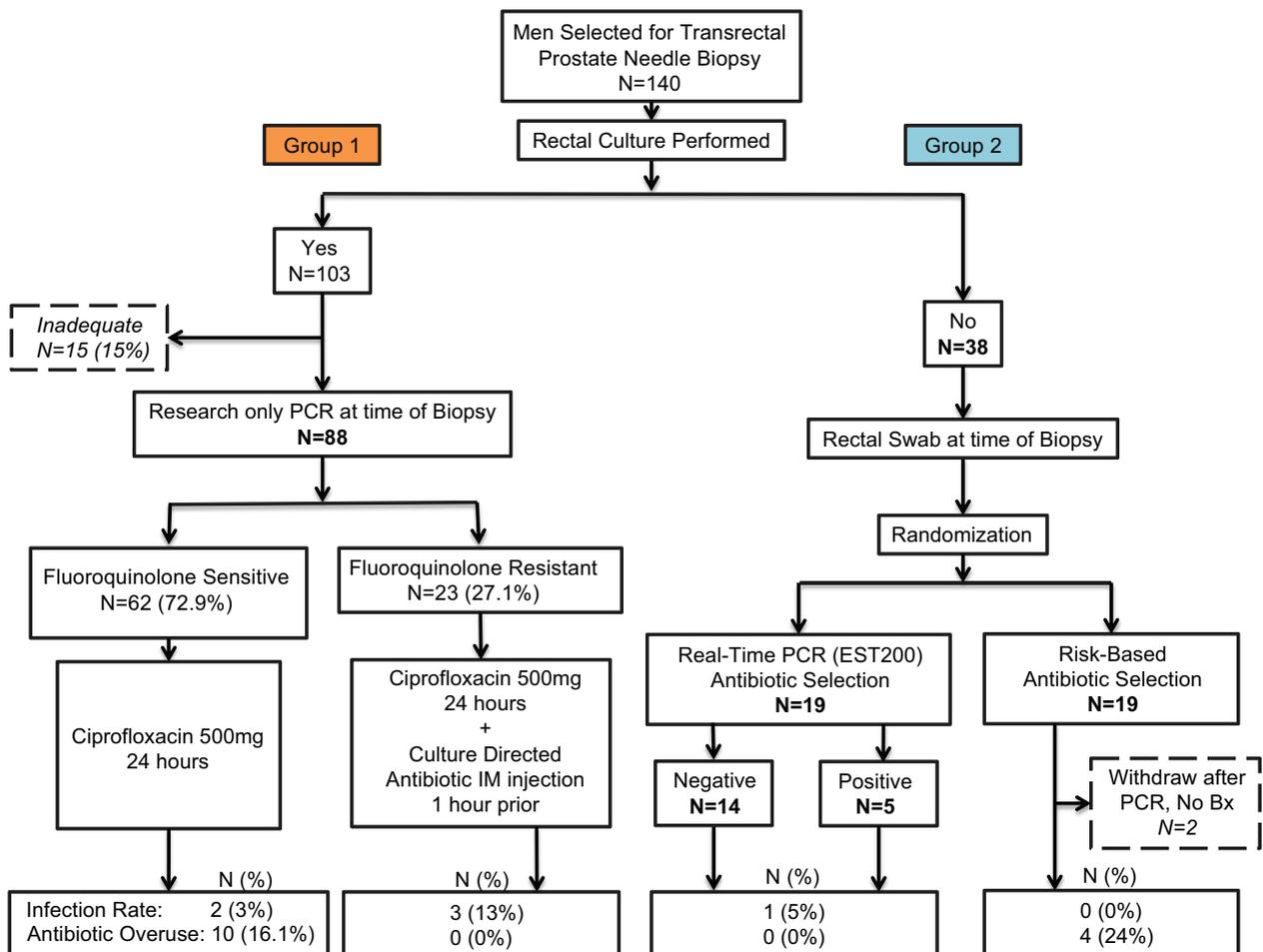


Figure 1. Consort flow diagram. We display a consort flow diagram to describe enrollment, comparisons and proportion of patients randomized to a particular treatment strategy. (Color version available online.)

(intervention group), the patient was randomized to either physician choice based on risk factors or the PCR results using a predetermined algorithm (previously published).⁸ Afterward, patients in both groups undergo a chart review and phone questionnaire in 10 days to inquire about infectious complications.

Rectal Swab Culture (study reference standard): We formally tested 1 culturette on ciprofloxacin infused (10 mg/L) MacConkey agar at the South Texas Healthcare Laboratory to serve as the study gold standard. The technologist selected a representative culture of each distinct colonial morphotype for identification and susceptibility using the Vitek 2 instrument (bioMerieux, Durham, NC) GN identification cards based on the Clinical and Laboratory Standards Institute interpretative criteria.⁹

EST200 PCR: We took the second culturette to our research lab for real-time qPCR analysis. The swab tip was resuspended in 300 μ L of sterile water, heated for 10 minutes at 98°C, and centrifuged at 10,000 rpm for 2 minutes. We used the supernatant in qPCR on Rotor-Gene Q instrument (Qiagen, Hilden, Germany) in 10- μ L reaction volume with 1 μ L of sample added as follows: (a) presence and *E coli* DNA load in the fecal sample was detected with *uidA*-specific primers (PrF 5'-tcttgcgcttttcgctgga, PrR 5'-cagcgcgtatgtattgccc) using Rotor-Gene SYBR-Green qPCR (Qiagen); (b) specific probes for *E coli* clonal groups ST131, its subclone ST131-H30, and ST69 were used to detect respective *E coli* clones in a multiplex reaction using QuantiFast Probe PCR Kit (Qiagen). We calculated sensitivity, specificity, negative predictive value, positive predictive value, and an area under the receiver operative curve (AUC).

Antibiotic Selection: In group 1, all patients continued their ciprofloxacin 500 mg orally every 12 hours (total of 2 doses). In group 2, the PCR test included identification of predominate *E coli* strains (ST131 and ST69). We obtained the specific resistance pattern from previous studies that have identified the most likely resistance pattern for each clone. The “clonal antibiogram” allowed for the predicted resistance rates of various bacteria from an existing database representing 2795 *E coli* isolated from clinical human urine samples collected over 5 years at hospitals worldwide.¹⁰ The clonal antibiogram—generated antibiotic resistance profile was used in the PCR group 2 to select particular antibiotics when randomized to intervention (previously published).¹¹

Statistical Analysis: Before the study, we identified that 33% of men presenting for TPB at the AMVA received additional to augment fluoroquinolone (FQ) prophylaxis based on physician choice. Our baseline FQR resistant rate from a rectal culture in the TPB population noted a 11 of 64 (17.1%) FQR and a 3% sepsis rate. Since the PCR test will not pick up every FQR

organism, we projected giving additional antibiotics to approximately 13% of patients. Our primary outcome is to reduce the use of antibiotics to the appropriate number of patients who present with at-risk colonization of FQR bacteria. Therefore, we anticipate a reduction in antibiotic use for TPB prophylaxis from 33% to 13% at our institution indicating about 138 patients needed. Our primary outcome is a reduction in dual antibiotic prophylaxis in the intervention versus usual care groups. Our secondary outcome is an infection or subsequent hospitalization. We use the Pearson's chi-squared test for both outcomes. We compare demographics with Student's *t* test or chi-squared, depending on the variable. Statistical analysis will be performed using SPSS v21 software.

RESULTS

Population: A total of 140 men participated in the study. Pre-biopsy cultures were utilized to guide prophylaxis in 103 men. Men without prebiopsy cultures were randomized to physician choice of antibiotic versus PCR-based approach ($n = 38$) (Figure 1). We did not reach our intended target of 138 patients randomized due to one several factors that included patient wait time, negative clinic logistics, and need for alternative antibiotics in the clinic (amikacin) due to patient allergies. Demographics are displayed in Table 1. Average clinic time was 67 minutes longer with PCR real-time testing ($P < .01$). In a PCR-based method, the randomization was equal ($n = 19$ PCR versus choice).

Antibiotic stewardship: Risk-based techniques may overcompensate with additional antibiotic augmentation (24% versus 0%, $P = .10$) compared to PCR- or culture-based approaches. Utilizing rectal culture-based targeted prophylaxis before biopsy continued to have 16% of patients to get gentamicin augmentation despite the culture noting no ciprofloxacin-resistant organisms.

Infections: We noted only 1 infection during the study which the PCR correctly identified the FQR ST131: H30+; however, a patient allergy led to an alternate antibiotic choice. PCR correctly identified 4 of 5 (80%) FQR specimens, which was an *E coli* strain.

Test Characteristics: We compared prebiopsy cultures against PCR to predict FQR status at the time of the biopsy (Table 2). The culture had a higher inadequate result compared to PCR (18% versus 2%, $P < .01$). Regarding predicting FQR at biopsy, prebiopsy cultures had an AUC of 0.91 (95%CI 0.84-0.99, $P = .04$) and PCR had an AUC of 0.68 (95%CI 0.55-0.82, $P = .01$) (AUC comparison; $Z = 2.7$, $P = .01$). Unfortunately, PCR missed 7 of 24 FQR noted at the time of biopsy, and a 15

Table 1. Cohort demographics

Demographic	Group 1 (N = 102) Culture N (%) or Median (IQR)	Group 2 (N = 38) PCR N (%) or Median (IQR)	P Value
Age	66 (60-70)	66 (62-70)	0.7
Race/Ethnicity			0.4
White	46 (45%)	17 (45%)	
Hispanic	28 (28%)	14 (37%)	
African-American	23 (23%)	7 (18%)	
BMI	30.6 (27.5-33.3)	30.5 (28.3-34.3)	0.5
Diabetes	27 (27%)	6 (16%)	0.2
Family history of prostate cancer	24 (24%)	11 (29%)	0.8
Prostate specific antigen	6.1 (4.4-8.4)	5.9 (3.4-9.1)	0.4
Abnormal prostate exam	30	12 (32%)	0.8

Table 2. Test characteristics

Test Characteristic	Pre-Biopsy Culture vs Day of Biopsy Culture N = 88	Day of Biopsy Culture vs Rapid PCR (EST200) N = 140
Sample size compared (N)	67	109
Inadequate Sample/Missing	21	31
Sensitivity	90.5%	51.2%
Specificity	91.3%	87.9%
Positive predictive value	82.6%	39.5%
Negative predictive value	95.5%	73.3%
AUC (confidence interval)	0.91 (0.82-0.99)	0.68 (0.55-0.82)
P value	0.04	0.01

of 56 (26.8%) were falsely positive if you take any of the PCR primers being positive. For instance, if any of the ST131, H30, or ST69 were present, the test would be considered positive. The PCR test attained an AUC of 0.68 (95%CI 0.55-0.82, $P = .01$) for detection of total FQR on the day of the biopsy.

DISCUSSION

The EST200 POC test needs substantial enhancement to improve sensitivity and specificity but does have a moderate ability to detect FQR and enhance antimicrobial stewardship. The study did not complete full enrollment and is underpowered; however, our results indicate preliminary data that a POC test may be able to assist providers with antibiotic decision-making while utilizing the least amount of antibiotics but will require substantial enhancements prior to another clinical trial. Our study did not have the PCR test capabilities directly in office; therefore, the samples were immediately taken to our lab which was an approximate 5-minute walk. We then communicated directly as soon as the results were available. This approach did take a coordinator to transport the samples and a lab tech to run the samples and report. Our goal in the future is to take our technology to a point of care test similar to a rapid streptococcus test in a primary care physicians office. We needed this current study to identify the current barriers such as length of time to run the test among other logistical concerns to move forward to larger studies.

The current clinical paradigm to protect men from prostate biopsy infection is to administer a fluoroquinolone antibiotic for less than 24 hours the day of the procedure.¹ Physicians have approached this issue with several different solutions for addressing this concern in clinical practice. First, identifying individuals at high-risk for infection and providing only them with additional antibiotics (option A) or provide fluoroquinolone plus an additional antibiotic to all patients undergoing a biopsy (option B). The risk-based approach (option A) usually leads physicians to give about 30% of patients either gentamicin or a cephalosporin. With the "augmentation for all" approach (option B), the physician provides 100% of patient's additional antibiotics.³ Another strategy includes rectal culture or "targeted prophylaxis." Targeted prophylaxis (option C) could be used to either replace a fluoroquinolone with a different antibiotic or to

augment fluoroquinolone antibiotics with a second antibiotic selected from the resistance profile if fluoroquinolone-resistant bacteria are isolated. Fluoroquinolones are given despite noting FQR because several studies have identified infections with FQ sensitive organisms when the antibiotic is changed and FQ is not given.^{12,13} Lastly (option D) is to avoid the rectum and perform a transperineal prostate biopsy. Our study seeks to provide the initial data to change the clinical paradigm and reduce overuse of antibiotics while maintaining infection control by using a POC test.

We targeted ST131 *E coli* because our group previously identified that more than 70% of prostate biopsy infections leading to hospitalization were of a single clonal group of ST131 *E coli*.⁷ Therefore, we predicted an AUC of 0.70 for our point of care test, and we achieved an AUC of 0.68 ($P = .04$). We acknowledge for a POC test to be more useful to physicians, it would need to obtain a much higher AUC. Targeting the entire fluoroquinolone-resistant bacteria population would be closer to the rectal culture using ciprofloxacin infused MacConkey agar as the gold standard. Unfortunately, the study gold standard culture only achieved an AUC of 0.91 compared to cultures performed the day of the biopsy. We also understand the rectal culture—targeted prophylaxis techniques do not protect all patients.¹²

Arguments against targeted prophylaxis discussed by urologists at the AUA Quality Improvement Summit 2014 included (1) failure to show the bacteria isolated from the rectum causing the infection, (2) the change in antibiotics is not recognized by the SCIP measure program and (3) obtaining a rectal culture prior to TPB is logistically inconvenient.⁶ Our group performed pulsed-field-gel electrophoresis (PFGE) of whole-cell DNA from *E. coli* bloodstream and rectal isolates. We demonstrated that whole-cell DNA PFGE pattern of the *E. coli* isolated from the blood during sepsis was identical to the PFGE pattern of the *E. coli* bacteria cultured from the rectum prior to TPB.¹⁴ This demonstrated that the infection-causing bacteria were in the rectum at the time of TPB and did not come from within the bladder or prostate itself. The last 2 problems with targeted prophylaxis (SCIP measures and logistics) are significant barriers for implementing pre-biopsy rectal cultures in clinical practice. Therefore, utilizing a fluoroquinolone-based protocol would keep the physician in compliance with recommended protocols and measures. Then using specified augmentation with the use of testing could provide

a targeted approach to antibiotic selection rather than all patients receiving augmented prophylaxis.

A significant concern is the escalation of additional broad-spectrum antibiotics to include intravenous antibiotics reserved for proven infections (eg, piperacillin/tazobactam) rather than prevention.¹⁵ The biopsy infection crisis may worsen the current critical issue of antibiotic resistance by increasing overall broad-spectrum antibiotic use for a single outpatient procedure. With limited new antibiotic development, antibiotic stewardship is a significant tool to preserve precious antibiotics.¹⁶ The unnecessary use of antibiotics will exacerbate the antibiotic resistance problem.¹⁷

In a recent statewide intervention to reduce prostate biopsy infections conducted by the University of Michigan, urologists chose between providing a patient with additional broad-spectrum antibiotics or obtaining rectal cultures to guide prophylaxis choices.¹⁸ Only 5% of patients enrolled were placed on the rectal culture-guided approach, presumably because obtaining culture results 1-2 weeks prior was inconvenient for providers and their patients. The use of additional broad-spectrum antibiotic was favored; therefore, antibiotic use increased from 23% prestudy to 88% during the study. Although the infection-related hospitalization rate decreased by 53% (1.19%-0.48%); this short-term success is likely to exacerbate antibiotic resistance in the long-term, placing emphasis on a POC test to curb antibiotic use so that it is only used by those at high risk.

Limitations of the study include the small sample size that did not reach our recruitment goal and therefore underpowered, which limits our data analysis and interpretation of the results. Once the standard of care became rectal culture after a significant infection, it became difficult to enroll men who did not already have a rectal swab noting presence or absence of FQR organisms. We did not have equipoise to randomized men with known colonization of FQR. We could not move sites as the PCR lab was set up within walking distance of only the Veterans Affairs (VA) urology clinic. We have identified the significant barriers in conducting a point of care test for prostate biopsy infection and currently are planning a follow-up large scale study after PCR test improvements identified in this trial including. We addressed previously in that we did not build the PCR to detect common FQR mutations and focused on sequence type due to previous data and we are expanding the PCR test.

CONCLUSION

Implementation of our PCR test lead to longer clinic time and would need to have broader inclusion of antibiotic resistance genes to improve predicting FQ resistance. Although we did not reach our accrual target we have identified opportunities to improve point of care testing. The test could continue to be modified to include faster result time, additional FQR specific genes, and potentially other sequence types.

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