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Original Article: Clinical Investigation

Impact of selective media for detecting fluoroquinolone-insusceptible/extended-spectrum beta-lactamase-producing *Escherichia coli* before transrectal prostate biopsy

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Abbreviations & Acronyms
CLSI = Clinical and Laboratory Standards Institute
E. coli = *Escherichia coli*
ESBL = extended-spectrum beta-lactamase
FQ = fluoroquinolone
LVFX = levofloxacin
MIC = minimum inhibitory concentration
PFGE = pulsed-field gel electrophoresis

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Objectives: To investigate the prevalence of fluoroquinolone-insusceptible and/or extended-spectrum beta-lactamase-producing *Escherichia coli* colonizing in the male rectum before transrectal prostate biopsy.

Methods: We carried out a prospective cohort study of men undergoing transrectal prostate biopsy. CHROMagar Orientation originally supplemented with levofloxacin and CHROMagar Orientation/extended-spectrum beta-lactamase were used for detecting fluoroquinolone-insusceptible and extended-spectrum beta-lactamase-producing *Escherichia coli*. Rectal specimens were collected before prostate biopsy, and the results of cultures in the selective medium were compared with drug susceptibility measured by standard methods. Targeted prophylactic antimicrobials were administered to patients with drug-resistant *Escherichia coli* and the incidence of postoperative prostatitis was investigated. In the case of prostatitis, pathogens preoperatively isolated from the rectum and those from urine were compared using pulsed-field gel electrophoresis.

Results: Rectal colonization of fluoroquinolone-insusceptible or extended-spectrum beta-lactamase-producing *Escherichia coli* was detected in 217 of 694 (31.3%) and 85 of 640 (13.3%) participants, respectively. The sensitivity and specificity of fluoroquinolone-insusceptible selective media were 96.8% and 88.2%, respectively. A total of 618 participants underwent transrectal prostate biopsy, and postoperative acute prostatitis was observed in four of 618 (0.6%) participants. *Escherichia coli* strains isolated preoperatively from the rectum and postoperatively from urine were found to be identical.

Conclusions: The present findings showed accuracy and performance of the selective media. Screening cultures before transrectal prostate biopsy using selective media seems to be helpful for guiding antibiotic prophylaxis and thus decreasing the rate of post-biopsy acute prostatitis.

Key words: extended-spectrum beta-lactamase, fluoroquinolone-resistant, prostate biopsy, screening, selective medium.

Introduction

Prostate biopsy is an essential procedure for the diagnosis of prostate cancer; however, acute bacterial prostatitis is one of the major adverse events in prostate biopsy, with reported rates of 0.1–7%.[1,2] According to the American Urological Association Best Practice Policy Statement, it is recommended that FQs and cephems be prophylactically administered before prostate biopsy. However, an increasing incidence of rectal colonization of FQ-resistant *E. coli* and ESBL-producing *E. coli* has been reported.[3–8]

Previous studies showed that rectal swabs and resistance profiles of the rectal flora are effective for targeting antibiotic prophylaxis and preventing post-transrectal prostate biopsy infections.[9,10] These findings led some groups to adopt rectal culture and directed prophylactic therapy before transrectal prostate biopsy. Thus, the use of selective media for detecting antimicrobial-resistant pathogens before transrectal prostate biopsy would be a possible preventive option for prostatitis after transrectal prostate biopsy.

The aims of the present study were to investigate the prevalence of FQ-insusceptible and/or ESBL-producing *E. coli* colonization in the male rectum before transrectal prostate biopsy using the selective solid media, and to evaluate the accuracy of the selective media compared with the results of standard drug-susceptibility testing. Furthermore, prophylactic antimicrobials administered according to the presence of colonization of FQ-insusceptible or ESBL *E. coli*, the incidence of postoperative febrile urinary tract infection, and homology between pairs of pathogens isolated preoperatively from the rectum and postoperatively from urine samples were evaluated.

Methods

Patients

We carried out a prospective cohort study of Japanese men undergoing transrectal prostate biopsy at five institutions located in Okayama: Okayama University Hospital, Okayama Rosai Hospital, National Hospital Organization Okayama Medical Center, Okayama City General Medical Center and Japanese Red Cross Okayama Hospital. Between 1 July 2013 and 31 May 2015, rectal colonization of FQ-insusceptible and/or ESBL-producing *E. coli* of the patients, who were candidates for transrectal prostate biopsy, was examined. Patients with an allergic episode to FQ, an immunosuppressive status, a severe cardiovascular risk, an active urinary tract infection and a history of infection with FQ-insusceptible bacteria were excluded.

Ethics

This clinical study was approved by the Okayama University Institutional Review Board before study initiation (registration no. 1700). The study was registered with the UMIN Clinical Trials Registry, Japan (UMIN: R000015581) and has been completed. The participants reviewed the documents about this study, and received individual counseling followed by written consent.

Solid medium and incubation

CHROMagar Orientation (Becton Dickinson, Cockeysville, MD, USA) is a bi-plate that can allow differentiation of several bacteria by colony color; *E. coli* forms purple colonies.[11] To detect the target *E. coli* strains, two kinds of CHROMagar Orientation medium were developed by Kanto Chemical (Tokyo, Japan; Fig. 1): (i) CHROMagar Orientation medium originally supplemented with 0, 1, 2 or 4 μg/mL of levofloxacin for detection of FQ-insusceptible *E. coli*; and (ii) CHROMagar Orientation/ESBL, which has been already available, for detection of ESBL-producing *E. coli*.[12] According to a breakpoint published by CLSI, FQ-insusceptible in *E. coli* was defined as LVFX MIC ≥4 μg/mL.[13]

The rubber gloves used for digital rectal examinations carried out in outpatient clinics before admission were used for inoculation; streak with gentle pressure using a gloved finger on the selective media without overlapping. After the inoculation by smear, the solid medium was incubated for 24 h at 37°C in room air and the existence of purple colonies was judged.

Accuracy of selective media

To assess the accuracy of the medium, comparisons between the results of selective medium and the results of standard drug susceptibility testing recommended by CLSI were carried out.[13,14] For *E. coli* strains, the LVFX MIC of the strains isolated from the selective medium was measured by the broth microdilution method; for ESBL-producing *E. coli* strains, isolated strains were examined by the disc diffusion test.[13,14]

Clinical procedures

Before prostate biopsy, patients were given a 60-mL glycerin enema, and administered antimicrobial prophylaxis. Prophylactic antimicrobials administered to the patients were dependent on the clinicians in each hospital (e.g. a single-dose

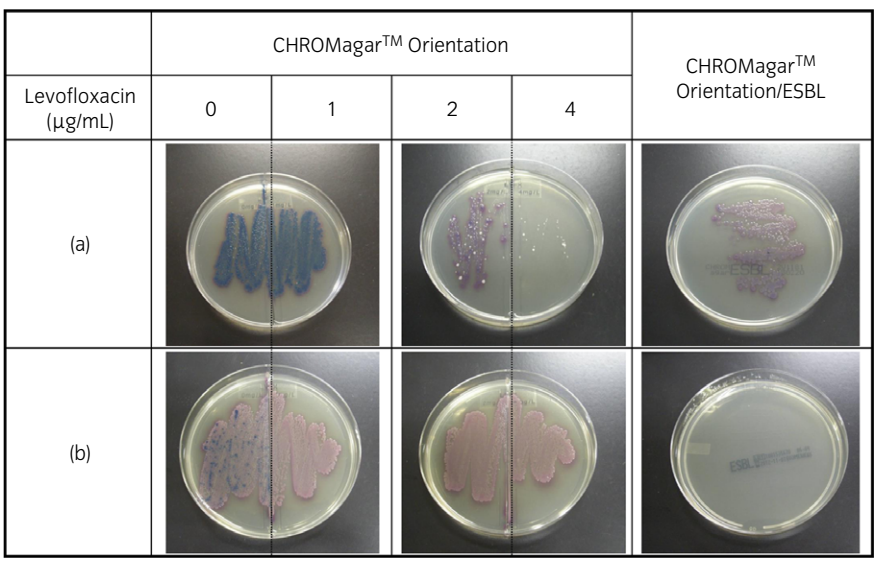

Fig. 1 Detection of *E. coli* using levofloxacin-including CHROMagar Orientation and CHROMagar Orientation/ESBL. Rectal specimens were screened by CHROMagar Orientation originally supplemented with LVFX and CHROMagar Orientation/ESBL, which were used for detecting antimicrobial-resistant *E. coli*. before transrectal prostate biopsy. (a) Plates from patients colonized with FQ-insusceptible *E. coli* (LVFX MIC 4 μg/mL) and ESBL-producing *E. coli*; (b) plates from patients colonized with FQ-resistant *E. coli* (LVFX MIC ≥8 μg/mL), but not ESBL-producing *E. coli*.

levofloxacin 500-mg tablet to patients without colonization of FQ-insusceptible or ESBL-producing *E. coli*; a single-dose sitafloxacin 100-mg tablet and a 200-mg single-dose intravenous injection of amikacin to patients with colonization of either FQ-insusceptible or ESBL-producing *E. coli*, in Okayama University Hospital). After disinfection of the rectum with povidone-iodine, 10 cores of prostate tissue were collected by transrectal ultrasonography-guided biopsy. After biopsy, rectal bleeding was stopped by pressure with a gloved finger for several minutes. The patients were discharged the next morning, and were asked in the outpatient clinic whether they experienced a febrile episode after biopsy. In addition, the clinical backgrounds of the patients and adverse events after prostate biopsy were examined.

Pulsed-field gel electrophoresis analysis

PFGE of *E. coli* isolates was carried out using standard procedures according to the manufacturer's protocol (Bio-Rad Laboratories K.K., Tokyo, Japan) as follows.[15] Briefly, isolates were grown in lysogeny broth medium at 37°C overnight. Bacterial cultures were embedded in 1.6% megabase agarose plugs (Bio-Rad Laboratories K.K.), lysed in lysozyme solution and digested separately with proteinase K solution. Size separation of the resulting DNA fragments was carried out for 18.5 h in 0.8% agarose gels using a CHEF DR-III apparatus (Bio-Rad Laboratories K.K.). A lambda ladder was used as a reference size standard. Pattern images were captured with a GelDoc XR (Bio-Rad Laboratories K.K.). The relatedness of each PFGE fingerprint was determined using Fingerprinting II (Bio-Rad Laboratories K.K.), and interpreted with the criteria proposed by Tenover *et al.*[16] In the dendrogram analysis, isolates with band patterns of 100% similarity were considered to have an identical type, and those with >80% similarity were considered to have a clonal relationship.

Results

Rectal culture

A total of 694 male patients were enrolled in the present study; 694 cultures were tested for detection of FQ-resistant *E. coli*, while 640 cultures were used for detection of ESBL-producing *E. coli* on each selective medium. In 54 patients, cultures for detection of ESBL-producing *E. coli* using CHROMagar Orientation/ESBL could not be carried out because of a preparative delay or putrefaction of the medium. From the rectal cultures, 217 of 694 (31.3%) contained FQ-insusceptible *E. coli*, and 85 of 640 (13.3%) had ESBL-producing *E. coli*. Regarding bacterial profiles, 60 of 193 strains of FQ-insusceptible *E. coli* (31.1%) were ESBL-producing, and 60 of 85 strains of ESBL-producing *E. coli* (70.6%) were FQ-insusceptible.

Accuracy of selective media

LVFX MIC was measured in 396 *E. coli* strains that were isolated from selective media; 181 strains were designated as FQ-insusceptible *E. coli* (isolated from 4 μg/mL of LVFX), and 215 strains as FQ-sensitive *E. coli*. The results of the evaluation of the accuracy of the levofloxacin-including CHROMagar Orientation are shown in Table 1. The rate of concordance of the FQ-insusceptible selective medium and broth microdilution method was 91.7% (363/396). By comparison with the results of the broth microdilution method, the sensitivity and specificity of the levofloxacin-including CHROMagar Orientation were 96.8% and 88.2%, respectively. Positive predictive and negative predictive values were 84.5% and 97.7%, respectively, showing a high accuracy. Of the 85 strains isolated as ESBL-producing *E. coli* through CHROMagar Orientation/ESBL, 65 could be examined, and 58 of 65 (89.2%) strains were defined as ESBL-producing *E. coli* through disc diffusion.

Antimicrobial prophylaxis and postoperative infectious complications

After the screening, 618 patients underwent transrectal prostate biopsy with antimicrobial prophylaxis shown in Figure 2. Postoperative prostatitis was observed in four of 618 (0.6%) patients, and all the pathogens isolated from urine samples were *E. coli*; three were FQ-resistant, and one was a FQ-resistant and ESBL-producing strain.

Table 1 Accuracy of the levofloxacin-including CHROMagar Orientation

	Broth microdilution isolates		
	MIC ≥4 μg/mL	MIC <4 μg/mL	Total isolates
CHROMagar Orientation isolates			
MIC ≥4 μg/mL	153	28	181
MIC <4 μg/mL	5	210	215
	158	238	396
Classification parameters			
Sensitivity	96.8%	False positive rate	11.8%
Specificity	88.2%	False negative rate	3.2%
Positive likelihood ratio	8.2	Positive predictive value	84.5%
Negative likelihood ratio	0.04	Negative predictive value	97.7%

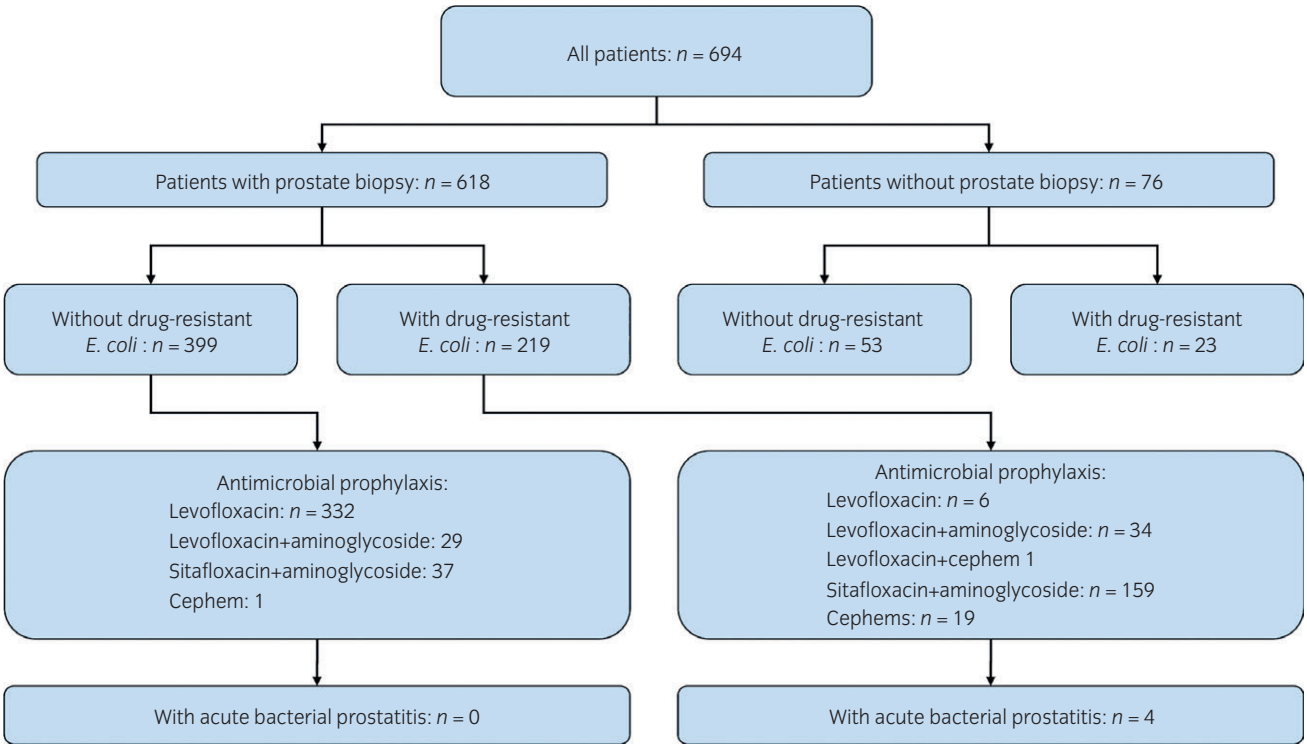

Fig. 2 Antimicrobial prophylaxis for transrectal prostate biopsy. The pathogen that caused postoperative prostatitis in patients with levofloxacin and amikacin was FQ-insusceptible and ESBL-producing *E. coli*.

PFGE analysis

E. coli strains were preoperatively isolated from the rectum and postoperatively from urine in two patients; these isolates were compared using PFGE. In the PFGE/dendrogram analysis, isolate band patterns from each patient were 95.7% and 91.3% similar, respectively, and were thus considered to have a clonal relationship (Fig. 3). In the drug-resistant analysis, patient A had FQ-insusceptible (LVFX MIC 4 μg/mL) and ESBL-producing *E. coli*. Patient B also had FQ-insusceptible (LVFX MIC 4 μg/mL) *E. coli*, but not ESBL-producing, strains.

Discussion

In the present study, the selective media had high accuracy based on a comparison with standard tests. In addition, relatively high numbers of FQ-insusceptible and ESBL-producing *E. coli* were isolated from men. Furthermore, in both of the evaluable patients, the pathogens causing postoperative febrile urinary tract infections were identical to strains isolated from the rectum before transrectal prostate biopsy.

A rising incidence of transrectal prostate biopsy infections as a result of drug-resistant bacteria is a matter of concern. The most common organism responsible for febrile urinary tract infections after transrectal prostate biopsy is *E. coli*.[7] Fecal carriage rates of FQ-resistant and ESBL-producing *E. coli* in patients who underwent transrectal prostate biopsy were 22% and 10.6%, respectively, in previous studies.[9,17] In the present study, high rates of FQ-insusceptible and ESBL-producing *E. coli* were observed (31.3% and 13.3%, respectively). The rectal flora might be the source of the antimicrobial-resistant strains, and thereby the cause of postoperative

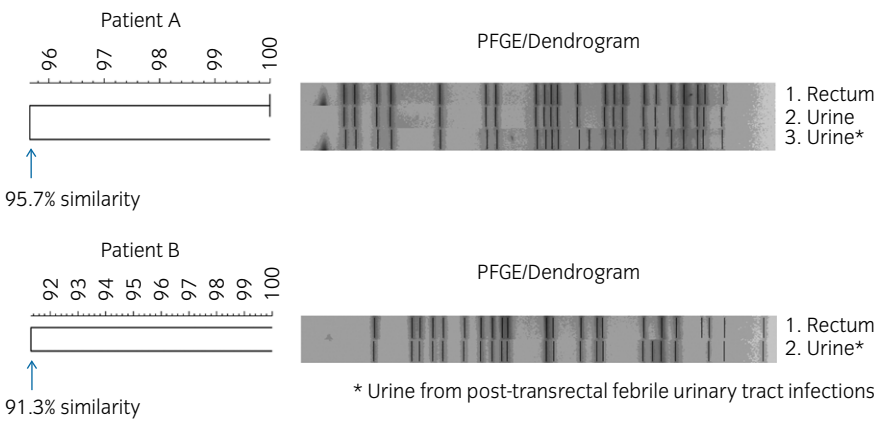

Fig. 3 Dendrogram of PFGE analysis. *E. coli* strains isolated preoperatively from the rectum and postoperatively from urine (patient A and patient B) were found to be identical through PFGE.

febrile urinary tract infections. Therefore, a logical and rapid approach to prevent infection is urgently required.

In the present study, we used selective media for detection of FQ-insusceptible and ESBL-producing pathogens from the male rectum. The use of selective media prevented the detection of false negative colonies; with standard media, a single colony isolated from a large number of colonies might be misinterpreted as a FQ-sensitive strain. The sensitivity and specificity of the selective media were better than those of media previously reported.[18] It is desirable to use an appropriate and targeted antimicrobial therapy, and in the present study, detection of drug-resistant *E. coli* using the selective media and accurate prophylaxis contributed to a low rate (0.6%) of postoperative bacterial prostatitis. On further analysis, the isolates preoperatively isolated from the rectum and those from urine had a clonal relationship as seen through PFGE. These results strongly suggest that screening of rectal flora before transrectal prostate biopsy should be carried out using selective media to guide prophylactic antibiotic treatment, with the goal of reducing postoperative bacterial prostatitis. In addition, the use of selective media is easier and more rapid than that of the traditional broth microdilution method and disc diffusion test.

According to an antibiogram in Okayama University Hospital, published in 2016, aminoglycoside is one of the most effective antimicrobial agents even for drug-resistant *E. coli* isolated from complicated urinary tract infection.[19] Thus, >60 patients with the drug-resistant *E. coli* strain were prophylactically administered levofloxacin in addition to amikacin, while two of them suffered from acute prostatitis. In contrast, one of the FQs, sitafloxacin, was given to 196 patients from whom FQ-insusceptible *E. coli* strains were isolated using the media, and any acute prostatitis was not observed. Sitafloxacin has stronger inhibitory activities against DNA gyrase and topoisomerase IV compared with conventional FQs, and sifloxacin has been shown to have a stronger bactericidal effect.[20] We have already reported the high susceptibility of sitafloxacin even for FQ-insusceptible *E. coli* strains isolated from patients with urinary tract infections.[21] Thus, sitafloxacin was selected in the present study for prophylaxis, and the present results showed a good susceptibility. According to the Japanese guidelines and the results of the present study, an antimicrobial prophylactic regimen for prostate biopsy might be recommended as follows: a single-dose levofloxacin 500-mg tablet for patients without risk; or single-dose sitafloxacin 100 mg or intravenous piperacillin/tazobactam 4.5 g for patients with drug-resistant *E. coli*.[22]

However, the present study has some important limitations. First, fecal carriage of FQ-insusceptible and ESBL-producing *E. coli* was assessed at a single point in time. Also, we examined only a single pathogen. Rectal culture and fecal microbiomes might not correspond well with those taken immediately before transrectal prostate biopsy. Second, we did not examine molecular typing or virulence genes. As Colpan *et al.* reported, the ST131 genotype, primarily its *H30* subclone, is present in most antimicrobial-resistant *E. coli*, and showed greater molecularly inferred virulence than did other *E. coli*.[23] Third, accuracy of the CO/ESBL was not shown in the present study, as it has been previously reported by Saito *et al.*; both the sensitivity and specificity were 100% and 93.3%, respectively.[24]

Further analysis, such as larger randomized controlled trials, will be necessary to evaluate the efficacy of the selective media for the reduction of postoperative acute bacterial prostatitis and sepsis. The present data suggest that screening cultures using selective media could identify patients at risk for post-transrectal prostate biopsy infections, and that culture-based targeted antimicrobial prophylaxis would be associated with lower rates of acute prostatitis.

Acknowledgments

This clinical study was approved by the Okayama University Institutional Review Board before study initiation (registration no. 1700). We thank all investigators who contributed to our study. We also extend our proud gratitude to Dr Tomoyasu Tsushima and Dr Ryuji Fujita.

Conflict of interest

None declared.

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