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Escherichia coli isolates from patients with bacteremic urinary tract infection are genetically distinct from those derived from sepsis following prostate transrectal biopsy

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ABSTRACT

Background: Transrectal ultrasound-guided (TRUS) prostate biopsy is a very common procedure that is generally considered relatively safe. However, severe sepsis can occur after TRUS prostate biopsies, with *Escherichia coli* being the predominant causative agent. A common perception is that the bacteria that cause post-TRUS prostate biopsy infections originate in the urinary tract, but this view has not been adequately tested. Yet other authors believe on the basis of indirect evidence that the pathogens are introduced into the bloodstream by the biopsy needle after passage through the rectal mucosa.
Methods: We compared *E. coli* isolates from male patients with bacteremic urinary tract infection (B-UTI) to isolates of patients with post prostate biopsy sepsis (PPBS), in terms of their sequence types, determined by multi-locus sequence typing (MLST) and their virulence markers.
Results: B-UTI isolates were much richer in virulence genes than were PPBS isolates, supporting the hypothesis that *E. coli* causing PPBS derive directly from the rectum. Sequence type 131 (ST131) strains and related strain from the ST131 were common (>30%) among the *E. coli* isolates from PPBS patients as well as from B-UTI patients and all these strains expressed extended spectrum beta-lactamases.
Conclusions: Our finding supports the hypothesis that *E. coli* causing PPBS derive directly from the rectum, bypassing the urinary tract, and therefore do not require many of the virulence capabilities necessary for an *E. coli* strain that must persist in the urinary tract. In light of the increasing prevalence of highly resistant *E. coli* strains, a new approach for prevention of PPBS is urgently required.

Article's main point: Bacteremic UTI isolates are much richer in virulence genes than are post-transrectal prostate biopsy sepsis isolates, supporting the hypothesis that *E. coli* causing post transrectal prostate biopsy sepsis derive directly from the rectum.

Introduction

Transrectal ultrasound-guided (TRUS) prostate biopsy is one of the most commonly performed procedures in urology. Although TRUS prostate biopsy is generally considered to be a relatively safe outpatient procedure, severe sepsis has been described in 0.1%–3.5% of cases after TRUS prostate biopsy, with *Escherichia coli* being the most commonly involved pathogen (Nam et al., 2010; Raaijmakers et al., 2002; Tal et al., 2003). Recently there has been an apparent increase in hospitalization for infectious complications after TRUS prostate biopsy (Loeb et al., 2011; Nam et al., 2010; Wagenlehner et al., 2013).

The role of antimicrobial prophylaxis in preventing infectious complications after biopsy is now well established (Zani et al., 2011), although life-threatening infections still occur despite prophylaxis (Carignan et al., 2012). Moreover, a systematic review and meta-analysis of randomized controlled studies confirms that prophylaxis reduces the rate of bacteriuria following prostate biopsy, although its effectiveness in reducing symptomatic UTI and bacteremia is less certain (Bootsma et al., 2008). The most commonly

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used prophylactic antimicrobials are the fluoroquinolones (Wagenlehner et al., 2013). Several publications have recently documented an alarming increase in resistance to these agents among *E. coli* in post-TRUS prostate biopsy infections (Feliciano et al., 2008; Zaytoun et al., 2011), as well as to other antimicrobial agents such as ampicillin, ampicillin/sulbactam, trimethoprim/sulfamethoxazole and gentamicin (Carignan et al., 2012); extended spectrum β-lactamase (ESBL)–producing isolates have been increasingly recovered (Briffaux et al., 2009). The rise over the years in resistance of *E. coli* to ciprofloxacin (up to 90%) is accompanied by a concomitant increase in the rate of post-TRUS prostate biopsy infectious complications (Carignan et al., 2012; Feliciano et al., 2008). If this trend continues, resistance will spread to other antimicrobial agents at increasing rates. An alternative preventive approach should thus be considered. For this purpose, it is important to inquire into the pathogenesis of post-TRUS prostate biopsy infections: what is the reservoir of the infecting organisms, what is the route of their acquisition, what is the virulence arsenal of the pathogens, etc.

It is a common perception that the bacteria responsible for post-TRUS biopsy infections originate in the urinary tract (Wagenlehner et al., 2013). It is therefore recommended to perform midstream urine culture prior to the procedure, and to choose the antimicrobial agent used for prophylaxis according to the susceptibility of the bacteria isolated from urine (Grabe et al., 2013). Yet other authors believe on the basis of indirect evidence that the pathogens are introduced into the bloodstream by the biopsy needle after passage through the rectal mucosa (Carignan et al., 2012; Williamson et al., 2012).

The aim of the present study was to compare *E. coli* strains responsible for post-TRUS prostate biopsy infections and *E. coli* from male patients with bacteremic urinary tract infection in terms of genetic virulence markers. Correctly identifying the source of post-TRUS prostate biopsy infections is an essential step in the development of more effective preventive measures.

Materials and methods

Patients

Patients with post-TRUS prostate biopsy sepsis (PPBS)

Twenty-one men with febrile sepsis due to *E. coli* following TRUS prostate biopsy were prospectively enrolled during the period of January 2010 through August 2012 at the E. Wolfson Hospital Holon, Israel. Inclusion criteria included recent TRUS prostate biopsy, fever (temperature, >38.0 °C), and positive blood and/or urine cultures for *E. coli*. Additionally, rectal samples for cultures were obtained from all patients after signing an informed consent. All patients presented symptoms within less than a week from the procedure, and over 90% presented them within 48 h of the biopsy.

Patients with bacteremic urinary tract infection (B-UTI)

During the same period, 24 men with bacteremic urinary tract infection due to *E. coli* were prospectively enrolled at the same institution. Inclusion criteria included male gender, fever (temperature, >38.0 °C), and positive blood and urine cultures for *E. coli*. None of the patients included in this study had a Foley catheter.

The E. Wolfson Hospital Research Ethics Committee approved the study protocol.

Blood, urine and rectal cultures

Blood samples were drawn aseptically from a peripheral vein. Each blood sample was inoculated into a set of blood culture bottles. Bottles were incubated in the BacT/Alert system (BioMerieux, Inc, Durham, NC) until flagged positive or for 7 days. A sample from a positive bottle was Gram stained and subcultured. A single *E. coli* colony was saved for further studies. Urine was cultured semiquantitatively on blood agar and cysteine–lactose–electrolyte–deficient agar plates using the calibrated loop technique. A single colony was retained for further study. Fecal swabs were inoculated on one quadrant of a MacConkey's agar plate, then streaked for isolation in the remaining three quadrants with a sterile loop and incubated overnight at 37 °C. Lactose- and indole-positive Gram-negative bacilli with a consistent colonial morphology similar to *E. coli* were identified. From each plate with Gram-negative bacterial growth a representative colony of each distinct morphotype (ranging from one to five morphotypes) was selected for identification using the Vitek 2 instrument (BioMerieux, USA) GN identification cards, and verified as *E. coli*. The *E. coli* isolate mix was subsequently re-streaked for isolation and transferred to the Department of Molecular Microbiology and Biotechnology, where a single colony was analyzed at random. Based on past studies (Lidin-Janson et al., 1978), this procedure has over 85% probability of identifying the dominant clone.

Table 1
Prevalence (%) of *E. coli* virulence genes typical to *E. coli* strains from male PPBS isolates versus B-UTI isolates (number of isolates noted in parentheses).

Gene	% Positive (PPBS) $N=20$	% Positive (B-UTI) $N=22$	FDR-corrected (p-value*)
focG	0 (0)	27 (6)	0.0310
sfa	0 (0)	32 (7)	0.0276
papC	10 (2)	50 (11)	0.0276
iroN	25 (4)	41 (9)	0.3376
malX	50 (10)	86 (19)	0.0310
ompT	65 (13)	91 (20)	0.0747

* Fisher's exact test, adjusted for multiple hypothesis by the Hochberg and Benjamini false discovery rate correction (Benjamini and Hochberg, 1995).

Serum survival growth assays

A single colony was picked and grown overnight in 2 ml of Davis minimal medium supplemented with 0.2% glucose. The overnight culture was then diluted 1:10 in 10 ml of Davis minimal medium supplemented with 0.4% glucose. After 3–4 h, when the cultures reached the end of logarithmic growth phase (O.D $_{595}$ of ~2) 20 μl of culture were transferred to 96-well microplates containing 40 μl of concentrated Davis minimal medium (×5) supplemented with 2% glucose in addition to either 140 μl of dd H_2O (control) or Human Serum (Sigma human serum H-4522, to 70% final concentration), to a final volume of 200 μl. *E. coli* K-12 MG1655 was used as negative control, since it is rapidly killed in the presence of serum, while avian pathogenic strain 789, which is highly serum-resistant (Ideses et al., 2005) was used as a positive control. The O.D. of the cultures in the plates were continuously measured in an ELX 800 Absorbance Microplate Reader (BioTek) at 37° with continuous shaking.

Virulence marker identification

Virulence genes presence (Table 1) was determined by PCR using primers from (Rodriguez-Siek et al., 2005), (Supplementary table S1). All PCR reactions were run in a Biometra T Personal thermocycler, using 25 μl reaction volumes. One microliter from 1:50 dilutions of overnight bacterial culture in ddH_2O pre-heated for 5 min. at 96 °C were used as template and 28 amplification cycles with an annealing temprature of 63°. Each reaction consisted of 0.2 μl Biotaq DNA Polymerase [5 U/μl, (Bioline)], 0.5 μl of 10 μM solution of the forward and reverse primers, 1.5 μl of 3 mM MgCl2, and a 2.5 μl dNTP mix of 2.5 mM each.