

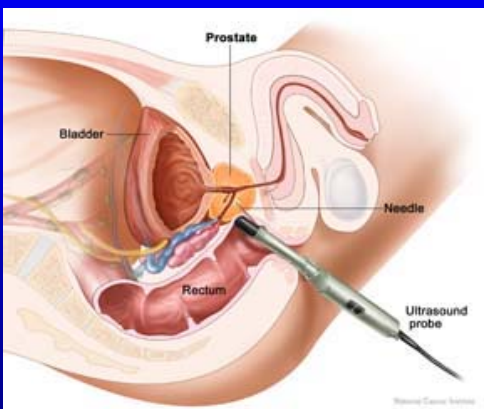
Detection of Fluoroquinolone Resistant Organisms from Rectal Swabs

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REVISED ABSTRACT

A transrectal prostate biopsy is one of the most common procedures performed by urologists and confirms the diagnosis of prostate cancer. The procedure is usually performed following antibiotic prophylaxes. When patients present with post-prostate biopsy infective symptoms, many are associated with fluoroquinolone resistant enteric pathogens. Therefore, the intestine/rectum is a likely source of the resistant organisms being introduced into the bloodstream at the time of the biopsy. The aim of this study was to develop a laboratory method to identify patients colonized with ciprofloxacin resistant enteric organisms and therefore at risk. Included in the study were 138 patients who were undergoing an ultrasound guided transrectal prostate biopsy. Prior to the biopsy, a rectal swab (Copan Diagnostics, Murrieta, CA) was collected and placed directly into 5 ml of brain heart infusion broth (BHI) containing 10 µg/ml of ciprofloxacin (Hardy Diagnostics, CA). Upon transport to the laboratory, the broth was incubated overnight at 35°C and subsequently subcultured onto MacConkey agar, MacConkey agar with 10 µg/ml of ciprofloxacin (Hardy Diagnostics) and HardyCHROM™ Coliform EC agar with 10 µg/ml of ciprofloxacin. Plates were incubated overnight at 35°C and examined for growth. All enteric gram-negative bacilli were characterized using the VITEK II (bioMérieux, Inc., NC); GN and AST-GN30 cards were used for identification and susceptibility testing, respectively. Upon subculture of the selective BHI broth, there was growth phenotypically resembling enterics on all three media from 30 (21.7%) patients. The quantity of organisms was similar on all plates. All 30 isolates were confirmed to be *E. coli* which were resistant to ciprofloxacin and levofloxacin with MICs of ≥4 µg/ml and ≥8 µg/ml, respectively. In conclusion, use of BHI broth containing ciprofloxacin for both transport and incubation of rectal samples facilitates the identification of patients harboring ciprofloxacin resistant enterics.



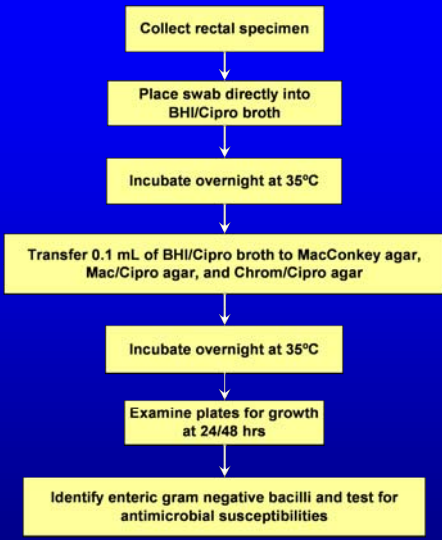
PURPOSE

To develop an isolation protocol to determine the incidence of fluoroquinolone resistant enteric gram-negative bacilli at the time of ultrasound guided transrectal prostate biopsy.

MATERIALS AND METHODS

After IRB approval (2008-6418), rectal swab specimens were collected from 138 patients prior to undergoing a transrectal ultrasound guided prostate biopsy from three separate institutions over a period of 16 months. 134 of the 138 patients received at least one dose (500 mg) of ciprofloxacin as a prophylactic within 24 hours prior to biopsy. The rectal swabs were obtained at the time of biopsy and fluoroquinolone resistant gram negative bacilli were recovered using selective media. The swabs were placed directly into five ml of brain heart infusion broth containing 10 µg/ml of ciprofloxacin and incubated overnight at 35°C in ambient air. Subsequently, the broth was subcultured to MacConkey agar, MacConkey agar with 10 µg/ml of ciprofloxacin, and HardyCHROM™ Coliform EC with 10 µg/ml of ciprofloxacin. The plates were inoculated by transferring 0.1 ml of broth onto the media and streaking for isolation. All three plates were incubated overnight at 35°C in ambient air. All enteric gram-negative bacilli were characterized on the Vitek II, using GN and AST-GN30 cards for identification and susceptibility testing, respectively.

PROTOCOL



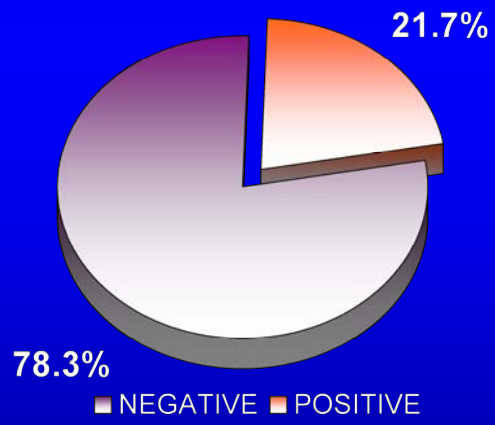
RESULTS

ID NO.	MacConkey	Mac/Cipro	Chrom/Cipro	CIPRO MIC	LEVO MIC
2	4+ <i>E. coli</i>	4+ <i>E. coli</i>	4+ <i>E. coli</i>	>=4	>=8
3	4+ <i>E. coli</i>	4+ <i>E. coli</i>	4+ <i>E. coli</i>	>=4	>=8
4	4+ <i>E. coli</i>	4+ <i>E. coli</i>	4+ <i>E. coli</i>	>=4	>=8
13	4+ <i>E. coli</i>	4+ <i>E. coli</i>	4+ <i>E. coli</i>	>=4	>=8
21	3+ <i>E. coli</i>	3+ <i>E. coli</i>	3+ <i>E. coli</i>	>=4	>=8
36	3+ <i>E. coli</i>	3+ <i>E. coli</i>	3+ <i>E. coli</i>	>=4	>=8
37	2+ <i>E. coli</i>	2+ <i>E. coli</i>	3+ <i>E. coli</i>	>=4	>=8
38	3+ <i>E. coli</i>	3+ <i>E. coli</i>	4+ <i>E. coli</i>	>=4	>=8
42	4+ <i>E. coli</i>	4+ <i>E. coli</i>	4+ <i>E. coli</i>	>=4	>=8
45	4+ <i>E. coli</i>	4+ <i>E. coli</i>	4+ <i>E. coli</i>	>=4	>=8
52	3+ <i>E. coli</i>	3+ <i>E. coli</i>	4+ <i>E. coli</i>	>=4	>=8
53	4+ <i>E. coli</i>	4+ <i>E. coli</i>	4+ <i>E. coli</i>	>=4	>=8
56	4+ <i>E. coli</i>	4+ <i>E. coli</i>	4+ <i>E. coli</i>	>=4	>=8
57	3+ <i>E. coli</i>	3+ <i>E. coli</i>	4+ <i>E. coli</i>	>=4	>=8
62	3+ <i>E. coli</i>	3+ <i>E. coli</i>	3+ <i>E. coli</i>	>=4	>=8
66	4+ <i>E. coli</i>	3+ <i>E. coli</i>	4+ <i>E. coli</i>	>=4	>=8
70	4+ <i>E. coli</i>	4+ <i>E. coli</i>	4+ <i>E. coli</i>	>=4	>=8
77	4+ <i>E. coli</i>	4+ <i>E. coli</i>	4+ <i>E. coli</i>	>=4	>=8
78	3+ <i>E. coli</i>	3+ <i>E. coli</i>	3+ <i>E. coli</i>	>=4	>=8
80	4+ <i>E. coli</i>	4+ <i>E. coli</i>	4+ <i>E. coli</i>	>=4	>=8
81	4+ <i>E. coli</i>	4+ <i>E. coli</i>	4+ <i>E. coli</i>	>=4	>=8
82	2+ <i>E. coli</i>	2+ <i>E. coli</i>	2+ <i>E. coli</i>	>=4	>=8
87	4+ <i>E. coli</i>	4+ <i>E. coli</i>	4+ <i>E. coli</i>	>=4	>=8
91	4+ <i>E. coli</i>	4+ <i>E. coli</i>	4+ <i>E. coli</i>	>=4	>=8
94	4+ <i>E. coli</i>	4+ <i>E. coli</i>	4+ <i>E. coli</i>	>=4	>=8
95	4+ <i>E. coli</i>	4+ <i>E. coli</i>	4+ <i>E. coli</i>	>=4	>=8
109	4+ <i>E. coli</i>	4+ <i>E. coli</i>	4+ <i>E. coli</i>	>=4	>=8
121	4+ <i>E. coli</i>	4+ <i>E. coli</i>	4+ <i>E. coli</i>	>=4	>=8
123	4+ <i>E. coli</i>	4+ <i>E. coli</i>	4+ <i>E. coli</i>	>=4	>=8
131	4+ <i>E. coli</i>	4+ <i>E. coli</i>	4+ <i>E. coli</i>	>=4	>=8

CAN BHI/CIPRO BROTH ALONE INHIBIT HIGH QUANTITIES OF NORMAL STOOL FLORA?

#	Normal Stool Flora	Broth to MacConkey	Broth to Mac/Cipro	Broth to Chrom/Cipro
1	1+	0	0	0
2	2+	0	0	0
3	3+	0	0	0
4	3+	0	0	0
5	4+	2 CFU	0	0
6	4+	32 CFU	0	0
7	4+	70 CFU	0	0
8	4+	50 CFU	0	0
9	4+	3+	0	0

In this experiment, stool specimens with normal enteric flora quantities of ≥ 4+ were the only ones showing breakthrough growth on regular MacConkey plates after being subcultured from BHI/Cipro broth. Therefore, when the quantity of normal stool flora is high enough, it can overcome the inhibitory effects of the BHI/Cipro broth.



CONCLUSION

- BHI broth containing 10 µg/ml of ciprofloxacin (BHI/Cipro) allows the recovery and enrichment of ciprofloxacin resistant enteric gram-negative bacilli from rectal swab specimens
- Selective agar containing ciprofloxacin used in conjunction with BHI/Cipro is necessary for patients with heavy normal stool flora
- There is no advantage using Chrom/Cipro agar over Mac/Cipro agar
- Using the described protocol, 21.7% of patients undergoing a prostate biopsy were colonized with ciprofloxacin resistant *E. coli*

SUMMARY

From the results obtained, BHI broth containing 10 µg/ml of ciprofloxacin used in conjunction with selective agar containing 10 µg/ml of ciprofloxacin is recommended for optimal recovery and selection of ciprofloxacin resistant enteric gram-negative bacilli