

## Tellurite resistance as a diagnostic marker for *Proteus-Morganella-Providencia* group infections.

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The *Proteus-Morganella-Providencia* group of bacteria includes a number of important human opportunistic pathogens. The extent of tellurite resistance phenotype among the PMP bacteria was investigated. Additionally, preliminary investigation was conducted into the potential use of screening for tellurite resistance as a rapid and efficient diagnostic test for the infections caused by the PMP bacteria. Twenty two clinical isolates of *Morganella* and *Providencia* displayed both low- and high-levels of potassium tellurite resistance, suggesting that the tellurite resistance phenotype is an integral feature of the PMP bacterial group. DNA sequences similar to the *ter* locus of *P. mirabilis* were identified by Southern blot hybridization in seven of these clinical isolates, which also displayed high level of tellurite resistance. It is hypothesized that at least three distinct  $Te^r$  systems, including that homologous to the *ter* locus, mediate tellurite resistance in the PMP bacteria. Based on the variable tellurite resistance levels and implied diversity of the underlying genetic determinants, routine screening for tellurite resistance appears to be an unsuitable basis for an informative diagnostic test of the PMP infections.

### INTRODUCTION

Tellurite has a long history as an antimicrobial agent for treatment of such conditions as leprosy, syphilis, tuberculosis, infections of the eye and seborrheic dermatitis (1). Antibacterial properties of potassium tellurite were reported by Alexander Fleming in the same paper that described bactericidal properties of penicillin (2). Throughout this century potassium tellurite has been used in selective media for the isolation of pathogens including *Corynebacterium diphtheriae*, *Staphylococcus aureus*, *Vibrio cholera*, verocytotoxigenic *Escherichia coli* O157 ("hamburger disease" bacterium) and *Shigella sonnei* (3, 4, 5).

The oxyanions of tellurium, tellurite and tellurate are highly toxic for most microorganisms, especially Gram-negative bacteria (6, 7), although some Gram-positive bacteria such as *C. diphtheriae*, *Enterococcus faecalis* and most *S. aureus* are naturally resistant (6). Tellurite toxicity is believed to be a conse-

quence of the oxidation of cellular thiols, with resulting shutdown of DNA and protein synthesis (7). However, the specific mechanism of toxicity is not yet known (10). Resistant bacteria produce jet-black colonies on solid media supplemented with potassium tellurite as the result of internal deposition of elemental tellurium (8, 9).

At least five chromosomal and plasmid-borne bacterial tellurite resistance systems have been described (10). However, these different determinants confer variable levels of resistance, show no sequence similarity at either DNA or protein level, do not share common genetic organization, and the number of genes within these  $Te^r$  operons varies greatly. Additionally, these systems are believed to mediate resistance by different yet unknown biochemical mechanisms (7).

Analysis of protein sequences from such databases as GenBank reveals that genes similar to the  $Te^r$ -determinants have been identified in a variety of unrelated eukaryotes and bacteria, including pathogens (10). This is unexpected, since very few microorganisms come into direct contact with Te-containing compounds in the environment. Thus, the emergence of several unrelated  $Te^r$  determinants among a wide range of bacterial spe-

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cies, including human pathogens, would suggest that these determinants provide some selective advantage in natural environments, which may be unrelated to the  $Te^r$  phenotype (8, 11).

Recently, molecular basis of tellurite resistance in *P. mirabilis* has been extensively characterized in our laboratory (12). Specifically, *P. mirabilis* chromosomal *ter* gene cluster have been cloned and sequenced. This work further demonstrated that the presence of potassium tellurite specifically activates the expression of these genes. Screening of the laboratory stock cultures and 31 clinical isolates of *P. mirabilis* and *P. vulgaris* in our laboratory demonstrated that tellurite resistance is both chromosomally encoded and present in all tested clinical isolates of *Proteus* spp. Since two previously characterized *ter* gene clusters of Gram-negative bacteria are plasmid borne (13, 9), we hypothesized that *P. mirabilis* may represent the evolutionary origin for dissemination of the plasmid-borne tellurite resistance loci among enteric pathogens (12).

*P. mirabilis* belongs to the *Proteus-Morganella-Providencia* (PMP) group of bacteria, which includes a number of important human opportunistic pathogens. These bacteria can colonize the urinary tracts and cause nosocomial infections in geriatric, psychiatric, and paraplegic patients, in individuals with structural abnormalities of the urinary tract, and in immuno-compromised and catheterized individuals in intensive-care units (14, 15,16).

In this study we have investigated the extent of tellurite resistance among the bacteria of the PMP group, and conducted preliminary investigation of the potential use of screening for tellurite resistance as a rapid and efficient diagnostic test for the infections caused by the PMP bacterial group.

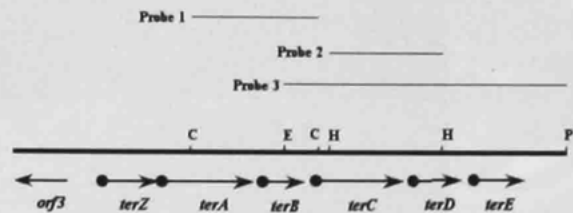
## METHODS

### Bacterial strains and media

Clinical isolates of the PMP group bacteria (16 *Morganella morganii* isolates, 5 *Providencia stuartii* isolates and a single *Providencia retgerri* isolate) were obtained from the laboratory of Dr. K. Forward (Department of Infectious Diseases), Victoria General Hospital, Halifax, Nova Scotia, Canada. *Proteus mirabilis* strain S2 was previously extensively characterized in our laboratory, and was used as the positive control for tellurite resistance. Fully tellurite sensitive *Escherichia coli* strain JF626 was used as the negative control. LB medium (BDH Inc., Toronto, ON) was used for all experiments. This medium was supplemented with potassium tellurite (5-250 mg/ml).

### DNA manipulations

The recombinant DNA techniques used were standard protocols (17). Plasmid DNA was isolated by alkaline extraction (19) and by Wizard<sup>®</sup> Plus SV Minipreps DNA Purification System according to the manufacturer's directions (Promega, Madison, WI). Restriction endonucleases were purchased from GIBCO BRL (Gaithersburg, MD), New Eng-



**Figure 1.** Physical map of the *ter* locus of *P. mirabilis* and the *ter*-specific probes. The map of the *ter* locus of *P. mirabilis* S2 is modified from the previous publication (19). Thin lines represent chromosomal DNA fragments excised with the corresponding restriction endonucleases and used subsequently as the hybridization probes (probes 1-3). The arrows below the restriction map indicate the predicted direction of transcription of the *ter* genes. Complete open reading frames are indicated by the arrows with solid circles, the simple arrow represents an incomplete ORF (*orf3*). Restriction enzyme sites: C, *Cla*I; E, *Eco*RI; H, *Hind*III; P, *Pst*I.

land Biolabs (Beverly, MA) and used according to the manufacturer's directions. DNA hybridization probes were generated by digestion with the appropriate restriction endonucleases recombinant plasmids containing partial *P. mirabilis* S2 *ter* loci (12) (Figure 1). Southern blot and colony blot hybridizations were performed using ECL<sup>®</sup> direct nucleic acid labeling and detection systems RPN 3000 kit (Amersham Pharmacia Biothec, Buckinghamshire, England) according to the manufacturer's directions. Oligonucleotides TERE-F (CGCTAATAAAAGCGCCAAGATG) and TERE-R (TTGTTGIAGWGGRATRTTTTG) were designed and synthesized previously (19).

## RESULTS

Screening of the PMP group clinical isolates for the ability to grow in the presence of potassium tellurite

Twenty-two clinical isolates of the PMP group bacteria were obtained from the laboratory of Dr. K. Forward. Table 1 describes the sites of isolation of the PMP strains that were tested during this work, and the results of the screening for the extent of tellurite resistance ( $Te^r$ ) among these strains. All strains were screened for the ability to grow in the presence of potassium tellurite in the concentration range from 5 mg/ml to 250 mg/ml. The  $Te^r$  levels of the tested strains were compared to those of *P. mirabilis* S2 and *Escherichia coli* JF626, which were used as a positive and negative control, respectively (12). When the growth medium was supplemented with the low concentration of tellurite (5 mg/ml), all of the tested strains were able to grow at the rates comparable to that of *P. mirabilis* S2. However, at high tellurite concentration (200 mg/ml and 250 mg/ml) the tested clinical isolates could be roughly separated in three groups, according to

**Table 1. Tellurite resistance levels among the clinical isolates of the PMP group.**

Clinical isolate	Strain #	Isolation site	Growth in the presence of potassium tellurite (mg/ml)*		
			5	200	250
<i>Morganella morganii</i>	1	Blood	+++ <sup>†</sup>	++	++
	2	Blood	+++	++	++
	3	Blood	+++	++	++
	4	Blood	+++	++	++
	5	Urine	+++	++	++
	6	Blood	+++	++	++
	7	Blood	+++	++	++
	8	Blood	+++	++	++
	9	P. D. Fluid	+++	++	++
	10	Blood	+++	++	++
	11	Abdominal distal	+++	+	+
	12	Urine	+++	++	++
	13	Urine	+++	++	++
	14	Urine	+++	++	++
	15	Urine	+++	++	++
	16	Abdominal proximal	+++	++	++
<i>Providencia stuartii</i>	1	Blood	+++	++	++
	2	Blood	+++	++	+
	3	Miscellaneous	+++	+++	+++
	4	Urine	+++	+/-	-
	5	Urine	+++	+++	+++
<i>Providencia rettgeri</i>	1	Blood	+++	-	-

\*PMP clinical isolates were plated on LB agar supplemented with potassium tellurite at an indicated concentration and incubated at 37°C. When growth medium contained 5 mg/ml of potassium tellurite, incubation continued for 2 days. LB agar plates supplemented with 200 and 250 mg/ml of potassium tellurite were incubated for a week.

<sup>†</sup>Bacterial growth was assessed using an arbitrary scale from "+++" to "--", where "+++" indicates that an entire plate was covered with bacterial growth following an incubation period (as was observed with *Proteus mirabilis*), and "--" indicated no bacterial growth following an incubation period (as was observed when fully sensitive *Escherichia coli* strain JF626).

their Te<sup>r</sup> levels. The first group included 16 *M. morganii* isolates and 2 *P. stuartii* isolates. These strains were able to grow in the presence of high tellurite concentrations, although their growth rates were below that of *P. mirabilis* S2. The second group consisted of 2 *P. stuartii* isolates (strains 3 and 5), which displayed high levels of tellurite resistance similar to that of *P. mirabilis* S2. The remaining *P. stuartii* strain 4 and a single *P. rettgeri* isolate were unable to grow in the presence of high tellurite concentrations and thus were included in the third group. These observations demonstrated that tellurite resistance phenotype is ubiquitous among the PMP bacterial group. However, different bacterial strains displayed different resistance levels.

The observed difference in the Te<sup>r</sup> phenotype may be a reflection of different underlying genetic mechanisms encoding the tellurite resistance phenotype.

#### Colony blot hybridization with the *ter*-specific probes

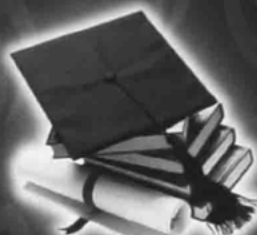
Colony clot hybridizations were performed to screen the PMP bacteria isolates for the presence of DNA sequences homologous to the *ter* locus. However, no conclusive data were generated by this approach due to high levels of non-specific hybridization. Specifically, every tested colony, including that of *E. coli* FJ626 (negative control) gave a strong hybridization signal (data not shown). The level of non-specific hybridization could not be minimized by using two different *ter*-specific probes (Figure 1, probes 1 and 2), increasing stringency of the washes and decreasing the exposure time for signal generation.

#### Amplification of the DNA sequences homologous to the *ter* locus by polymerase chain reaction

DNA sequences homologous to the *ter* locus were amplified by PCR with the TERE-F and TERE-R primers, using genomic DNA of the PMP clinical isolates as templates. These primers were previously designed based on the amino acid sequences of the known TerE and TerF proteins and *P.*



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