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Abstract

Potassium tellurite (K_2TeO_3) is an effective selective agent for O157:H7 Shiga toxin-producing *Escherichia coli* (STEC), whereas tellurite resistance in non-O157 STEC is variable with information on O45 minimal. High-level K_2TeO_3 resistance in STEC is attributable to the *ter* gene cluster with *terD* an indicator of the cluster's presence. Polymerase chain reactions for *terD* and K_2TeO_3 minimum inhibitory concentration (MIC) determinations in broth cultures were conducted on 70 STEC and 40 non-STEC control organisms. Sixty-six STEC strains (94.3%) were *terD*+ compared to 28 control organisms (70.0%; $P < 0.001$). The prevalence of *terD* in O103 STEC strains was 70%, whereas in all other serogroups it was ≥ 90%. The K_2TeO_3 geometric mean MIC ranking for STEC serogroups from highest to lowest was O111 > O26 > O145 > O157 > O103 > O12 1 = O45. The K_2TeO_3 geometric mean MIC was significantly higher in *terD*+ than in *terD*− STEC, but not in *terD*+ versus *terD*− control strains. Resistance to K_2TeO_3 (MIC ≥ 25 mg/L) was exhibited by 65/66 *terD*+ and 0/4 *terD*− STEC strains, compared to 12/28 *terD*+ and 8/12 *terD*− control strains. These results confirm previous studies showing the significantly higher prevalence of the *ter* gene cluster in STEC strains, and the relationship between these genes

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and K_2TeO_3 resistance in STEC and especially intimin (*eae*)-positive STEC, in contrast to non-STEC organisms. O45 and O121 STEC, although frequently *terD* positive, on average had significantly lower levels of K_2TeO_3 resistance than O26, O111, and O145 STEC.

Introduction

Strains of Shiga toxin-producing *Escherichia coli* (STEC) were estimated to have caused approximately 16% of all hospitalized cases of foodborne illness in the United States from 2009 to 2010 [18]. Ruminants serve as a major reservoir of STEC, and are the primary source of contamination of human food and water [8]. A subset of STEC classified as enterohemorrhagic *E. coli* (EHEC) causes hemorrhagic colitis and hemolytic uremic syndrome [8]. In recent years, over 90% of EHEC infections in the United States were due to seven serogroups, and the majority of the associated outbreaks were attributable to beef [7, 14]. The U.S. Department of Agriculture Food Safety and Inspection Service (FSIS) has declared these seven EHEC serogroups (O26, O45, O103, O111, O121, O145, and O157) to be adulterants in raw, non-intact beef [19].

Culture-based detection methods for non-O157 STEC, including those for the detection of FSIS adulterants, are relatively insensitive and in need of improvement. Several factors contribute to this insensitivity, but major ones are the diversity of the microorganisms targeted and the high levels of background microbiota in specimens [20]. While enrichment increases viable target cells in a sample, selective agents must inhibit the growth of background flora. In 1993, Zadik et al. [22] reported that K_2TeO_3 incorporation into agar media improved the selection of O157:H7 STEC. In 2000, Tarr et al. [15] reported the presence of a tellurite resistance and adherence-conferring island (TAI) on the chromosome of O157:H7 STEC. The TAI included four open reading frames that were homologous to tellurite-resistance (*ter*) genes carried on plasmids in *Alcaligenes* sp. (pMER610) and *Serratia marcescens* (pR478) [11, 15, 16, 21]. Bielaszewska et al. [3] and Orth et al. [13] reported that the *ter* gene cluster containing *terA-F* and *-Z* was correlated with growth of O26, and other non-O157 STEC, respectively, on K_2TeO_3- containing media. Essential to a functional *ter* operon, *terD* is a practical marker of its presence [10, 13, 17]. However, Kerangart et al. [12] recently reported that although the *ter* operon is

usually predictive of K_2TeO_3 resistance in non-O157 STEC this is not always the case, and more research on the STEC tellurite resistome will be required for the development of improved media for the recovery of STEC.

The objectives of this study were to determine the (1) prevalence of *terD* in a representative set of STEC strains and non-STEC control organisms; (2) minimum inhibitory concentration (MIC) of K_2TeO_3 for the same representative set of organisms; and (3) relationship between the prevalence of *terD* and the K_2TeO_3 MIC.

Materials and Methods

Bacterial Strains

One hundred ten bacterial strains, 70 STEC and 40 non- STEC controls, selected without knowledge of *terD* presence or absence, were included in this study. The STEC strains included ten of each USDA-FSIS adulterant serogroup (Table 1). The STEC strains were obtained from the American Type Culture Collection, Michigan State STEC Repository, collaborating investigators (D. H. Francis, J. B. Luchansky, T. G. Nagaraja, A. D. O'Brien, and D. G. Renter), and field isolates from other studies (Moxley). Thirty-eight, 30, one, and one of the STEC strains were of human, cattle, unknown, and food (hamburger) origin, respectively (Table 1). STEC strains were characterized for O-antigen and virulence genes by an 11-plex PCR [2], and represented a mixture of virulence genotypes with all positive for stx_1, stx_2 or both, and 68 positive for *eae*. Control strains were obtained from the University of Nebraska–Lincoln Veterinary Diagnostic Center (Table 2). These strains included both Gram-positive and Gram-negative bacteria, with most representing enteric microbiota, and especially members of the *Enterobacteriaceae* family. Records of the host species origins of the individual control strains were not available.

Polymerase Chain Reaction (PCR)

Bacterial strains stored at − 80 °C were streaked for isolation onto 5% sheep blood agar plates (Remel, Lenexa, Kansas; BAP) and incubated at 37 °C for 24 h. A DNA template was prepared for each strain

Table 1 Minimum inhibitory concentrations (MIC) for Ko_2TeO_3 and polymerase chain reaction assay results for *terD* in representative STEC strains

Strain	Origin[a]	Serotype[b]	*stx*$_1$ [b]	*stx*$_2$ [b]	*eae*[b]	*terD*[b]	MIC[c]	Resistance[d]
DEC10B	H	O26:H11	+	−	+	+	282.84	+
97-3250	H	O26:H11	+	+	+	+	141.42	+
DA-10	H	O26:NM	+	−	+	+	141.42	+
16272	C	O26	+	−	+	+	282.84	+
1577-88	C	O26:H11	+	−	+	+	100.00	+
H30	H	O26:H11	+	−	+	+	200.00	+
2003-3014	H	O26:H11	+	+	+	+	141.42	+
DEC10e	C	O26:H11	+	−	+	+	200.00	+
413/89-1	C	O26:H11	+	−	+	+	200.00	+
IHIT2087	C	O26:H11	+	−	+	+	141.42	+
DA-21	H	O45:H2	+	−	+	+	50.00	+
DEC11C	H	O45:H2	+	−	+	+	35.35	+
MI01-88	H	O45:H2	+	−	+	+	50.00	+
B8026-C1	C	O45:H2	+	−	+	+	12.50	−
B8227-C8	C	O45:H2	+	−	+	+	282.84	+
CDC 96-3285	H	O45:H2	+	−	+	+	141.42	+
2000-3039	H	O45:H2	+	−	+	+	25.00	+
1.2622	C	O45:H12	+	−	−	−	4.42	−
D88-28058	C	O45:NM	+	−	+	+	100.00	+
B8228-C2	C	O45:H2	+	−	+	+	50.00	+
MT#80	H	O103:H2	+	−	+	−	3.12	−
TB154A	H	O103:H2	+	−	+	−	3.12	−
8419	H	O103:H25	+	−	+	+	50.00	+
15612-1	C	O103:H11	+	−	+	+	282.84	+
CDC 90-3128	H	O103:H2	+	−	+	+	282.84	+
2006-3008	U	O103:H11	+	−	+	+	200.00	+
236-5	C	O103	+	+	+	+	200.00	+
6:38	H	O103:H2	+	−	+	+	200.00	+
RW1372	C	O103:H2	+	−	+	−	6.25	−
89-118	C	O103:NM	+	−	+	+	282.84	+
RD8	H	O111:H10	−	+	−	+	141.42	+
3215-99	H	O111:H8	+	+	+	+	200.00	+
0201 9611	H	O111:H8	+	−	+	+	200.00	+
7726-1	C	O111	+	+	+	+	200.00	+
8266-1	C	O111	+	+	+	+	141.42	+
10049	C	O111:H11	+	−	+	+	400.00	+
JB1-95	H	O111	+	+	+	+	200.00	+
ATCC BAA-2440	H	O111:H8	+	+	+	+	282.84	+
DEC8b	C	O111:H8	+	+	+	+	282.84	+
IHIT1703	C	O111:H2	+	−	+	+	200.00	+
DA-5	H	O121:H19	−	+	+	+	35.36	+
MT#2	H	O121:H19	−	+	+	+	50.00	+
DA-37	H	O121:H19	−	+	+	+	25.00	+
E191F-1	C	O121:H19	−	+	+	+	70.71	+
KDHE 55	H	O121	−	+	+	+	25.00	+
CDC 97-3068	H	O121:H19	−	+	+	+	50.00	+
2002-3211	H	O121:H19	−	+	+	+	50.00	+
3377-85	H	O121:H19	−	+	+	+	70.71	+

Table 1 (continued)

Strain	Origin[a]	Serotype[b]	*stx*$_1$ [b]	*stx*$_2$ [b]	*eae*[b]	*terD*[b]	MIC[c]	Resistance[d]
8-084	C	O121:H19	–	+	+	+	50.00	+
KDHE 47	H	O121	–	+	+	+	50.00	+
860B	C	O145	–	+	+	+	200.00	+
GS G5578620	H	O145:H28	+	–	+	+	282.84	+
IH 16	H	O145:NM	–	+	+	+	141.42	+
1234-1	C	O145:H28	+	+	+	+	200.00	+
7744	C	O145	+	–	+	+	25.00	+
83-75	H	O145:NM	–	+	+	+	282.84	+
99-3311	H	O145:NM	+	+	+	+	100.00	+
B6820-C1	C	O145:NM	–	+	+	+	50.00	+
IHIT0304	C	O145:H28	–	+	+	+	200.00	+
KDHE 53	H	O145	+	+	+	+	282.84	+
86-24	H	O157:H7	–	+	+	+	35.35	+
S2006 #1	C	O157:H7	+	+	+	+	141.42	+
S2006 #2	C	O157:H7	+	+	+	+	100.00	+
S2006 #3	C	O157:H7	+	+	+	+	141.42	+
S2006 #4	C	O157:H7	+	+	+	+	100.00	+
B2387	H	O157:H7	–	+	+	+	141.42	+
1:361	H	O157:H7	–	+	+	+	100.00	+
9:100	H	O157:H7	–	+	+	+	70.71	+
93-111	H	O157:H7	+	+	+	+	100.00	+
EDL 933	F	O157:H7	+	+	+	+	50.00	+
Mean ± SE[e]							91.92 ± 1.02	

a. *H* human (clinical), *C* cattle (feces), *U* unknown, *F* food (hamburger)

b. Serotype based on molecular and/or genotypic (PCR) characterization; H-type was not determined for some strains and in this case, left blank, or listed as NM, non-motile if known; Shiga toxin gene (*stx*) subtype; 1, *stx*1; 2, *stx*2. Presence (+) or absence (–) of intimin (*eae*) and tellurite-resistance (*terD*) genes

c. Geometric mean calculated from log2- transformed K2TeO3 MIC (mg/L); data from two experiments

d. Resistant (+) and susceptible (–) strains based on mean K2TeO3 MIC of ≥ 25 and < 25 mg/L, respectively

e. Geometric mean ± standard error K2TeO3 MIC (mg/L) of all STEC strains in study (n = 70)

by suspending an individual isolated colony in 50 µL of ultrapure water and heating at 95 °C for 10 min. Individual PCR reactions were conducted using a Bio-Rad T100™ Thermal Cycler (Bio-Rad, Hercules, CA). Primers targeting *terD* (Eurofins MWG Operon) and reaction conditions were as described by Taylor et al. [17]. Each 25-µL PCR reaction contained 2.5 µL of 10x ThermoPol buffer, 0.5 µL of dNTP mix, 0.5 µL of *Taq* polymerase (New England BioLabs, Ipswich, MA), 2.0 µL of each primer (forward and reverse), and 2.0 µL of the appropriate DNA template. PCR products were visualized by using either a QIAxcel

Table 2 Minimum inhibitory concentrations (MIC) for K_2TeO_3 and polymerase chain reaction (PCR) assay results for *terD* in non-STEC control organisms

Organism name (strain)	MIC[a]	Resistance[b]	*terD*
Aeromonas hydrophila	12.50	−	+
Citrobacter freundii	0.78	−	+
Corynebacterium renale	25.00	+	−
Enterobacter cloacae	0.78	−	+
Enterococcus faecalis	> 400.00	+	+
Escherichia coli (8863-88-1)	6.25	−	−
Escherichia coli (ATCC 25922)	0.20	−	−
Escherichia coli (11182-2)	6.25	−	+
Escherichia coli (15195-2)	6.25	−	+
Escherichia coli (16118-2)	6.25	−	+
Escherichia coli (17298-2)	4.42	−	+
Escherichia coli (2534-86)	1.10	−	+
Escherichia coli (G58-1)	6.25	−	+
Escherichia vulneris	100.00	+	+
Klebsiella pneumoniae	3.12	−	+
Kosakonia cowanii	12.50	−	−
Listeria monocytogenes	200.00	+	+
Micrococcus luteus	100.00	+	+
Morganella morganii	6.25	−	+
Pantoea sp.	400.00	+	−
Pasteurella multocida	200.00	+	+
Proteus mirabilis	> 400.00	+	+
Proteus vulgaris	400.00	+	−
Providencia rettgeri	0.20	−	−
Pseudomonas aeruginosa	35.36	+	+
Pseudomonas koreensis	100.00	+	−
Rhodococcus equi	400.00	+	−
Salmonella ser. Choleraesuis	100.00	+	+
Salmonella ser. Gallinarum	35.36	−	+
Salmonella ser. Pullorum	6.25	−	+
Salmonella ser. Typhimurium	1.56	−	+
Serratia marcescens	0.20	−	+
Staphylococcus aureus	> 400.00	+	+
Staphylococcus epidermidis	100.00	+	−
Staphylococcus intermedius	100.00	+	+
Streptococcus gallolyticus (VDC 10941-01-2)	400.00	+	+
Streptococcus gallolyticus (VDC 21353-05-1)	> 400.00	+	+
Streptococcus uberis	100.00	+	−
Yersinia enterocolitica	0.55	−	+
Yersinia ruckeri 25.00	+	−	
Mean ± SE[c]	19.82 ± 3.86		

a. Geometric mean calculated from log2- transformed K2TeO3 MIC (mg/L) based on data from two experiments

b. Resistant strains (+) have mean K_2TeO_3 MIC ≥ 25 mg/L. Susceptible strains (−) have mean tellurite MIC < 25 mg/L

c. Geometric mean ± standard error K_2TeO_3 MIC (mg/L) of all control strains in study (n = 40)

Table 3 Mean K_2TeO_3 minimum inhibitory concentrations (MIC) and tellurite-susceptible or -resistant status of *terD*− versus *terD*+ STEC strains and control Organisms

	STEC		Control organisms	
	terD−[a]	*terD*+[a]	*terD*−	*terD*+
MIC mean ± SE[b]	4.05 ± 1.09	111.07 ± 1.01*	26.54 ± 1.17	17.47 ± 1.09
Number of strains tested	4	66	12	28
Number susceptible[c]	4	0	4	16
Number resistant[d]	0	66	8	12

a. *terD* negative (*terD*−) or *terD* positive (*terD*+) based on PCR results

b. Geometric mean ± standard error mean calculated from log2- transformed MIC (mg/L) based on data from two experiments. Asterisk denotes significant difference ($P < 0.05$) between *terD*− and *terD*+ strains

c. Number of strains susceptible to K_2TeO_3, designated by MIC < 25 mg/L

d. Number of strains resistant to K_2TeO_3, designated by MIC ≥ 25 mg/L

automated capillary electrophoresis instrument and the QIAxcel DNA Screening Kit (Qiagen, Hilden, Germany) or on an ethidium bromide stained 2% agarose gel. O157:H7 STEC strain EDL 933 was used as a positive control for the presence of *terD*, and 2 µL of ultrapure water was used in place of DNA template as the negative control. For each strain tested, two independent PCR experiments, consisting of two technical replicates each, were conducted.

Minimum Inhibitory Concentration (MIC)

Cation-adjusted Mueller–Hinton broth (CAMHB) was used as both diluent and liquid growth media in this experiment (Becton, Dickinson and Company, Franklin Lakes, NJ). Serial two-fold dilutions of solution were carried out resulting in 14 different tellurite concentrations ranging from 0.1 to 800 mg/L; a solution of CAMHB with no K_2TeO_3 was used for control purposes. Ninety-six well microtiter plates containing replicates of 50 µL of a concentration per well were used to conduct MICs. Plates prepared prior to inoculation were sealed and stored at −20 °C until used.

Each strain was streaked for isolation on BAP and incubated overnight at 37 °C. Individual colonies were picked into 5 mL of Sensititre™ demineralized water (Thermo Scientific, Waltham, MA) to achieve a bacterial suspension equivalent to 0.5 McFarland unit (approximately 10^8 CFU/mL) as determined by a Sensititre™ AutoInoculator (Thermo

Scientific, Waltham, MA). 10 µL of each bacterial suspension was added to 11 mL of Sensititre™ CAMHB w/TES [*N*-tris(hydroxymethyl) methyl-2-aminoethanesulfonic acid] (Thermo Scientific, Waltham, MA). 50 µL of the final inoculated CAMHB was added to a well of the thawed 96-well plate with the exception of the negative control wells. The inoculation format resulted in two wells per strain per K_2TeO_3 concentration. Culture purity was confirmed using a subsample from the inoculated positive control wells. All plates were incubated at 37 °C for 18 h. A Sensititre™ Manual Viewbox (Thermo Scientific, Waltham, MA) was used to determine the presence or absence of growth. Visualized growth was scored as positive. For each strain tested, two technical replicates were performed for each of the two independent MIC experiments. The MIC of a strain was determined as the lowest tellurite concentration that inhibited growth in both technical replicates in an experiment [5]. The $\log_2$- transformed MIC of each strain in each experiment and the geometric mean MIC of each strain using the $\log_2$- transformed data from both experiments was determined. In the case of MICs ≥ 400 mg/L, a value of 400 mg/L was used for the $\log_2$ transformation to allow for calculation of a geometric mean. The geometric mean and standard error for each STEC serogroup, all STEC combined, and all control strains combined were determined from the $\log_2$- transformed data from individual strains. The minimum concentration required to inhibit growth of 90% of strains tested in each serogroup was determined as the MIC_{90} [5].

Statistical Analysis

All statistical analyses were performed using JMP Pro Version 10.0 (SAS Institute, Cary, NC). An analysis of means for proportions using an adjusted Wald test was used to analyze the proportions of STEC and control organisms that tested positive for *terD* (*terD*+) by PCR. The Student's *t* test was used to determine whether the geometric mean MIC for the following group comparisons were significantly different using α at 0.05: (1) STEC isolates versus control isolates; (2) *terD*+ STEC versus *terD*-negative (*terD*−) STEC; and (3) *terD*+ controls versus *terD*− controls. A one-way analysis of variance (ANOVA) followed by Tukey–Kramer HSD analysis was conducted on geometric means of K_2TeO_3 MIC values for comparison at the serogroup level using α at 0.05.

Statistical analyses for agreement between *terD* PCR and K_2TeO_3 MIC results were performed by using Bowker's test to determine if there was significant symmetry of disagreement between the two tests. If Bowker's test was > 0.05, Cohen's kappa coefficient (κ) was used to determine the level of agreement using the following criteria: if $\kappa \leq 0.20$ = poor; if $0.21 \leq \kappa \leq 0.40$ = fair; if $0.41 \leq \kappa \leq 0.6$ = moderate; $0.61 \leq \kappa \leq 0.80$ = good; and $0.81 \leq \kappa \leq 1.00$ = very good [1]. Isolates were classified as susceptible (< 25 μg/mL) or resistant (≥ 25 μg/mL) based on K_2TeO_3 MIC. Presence or absence of *terD* was based on PCR analysis. The relationship between the presence of intimin (*eae*) and K_2TeO_3 resistance was examined using Pearson's χ^2 test.

Results

Sixty-six of 70 STEC strains (94.3%) were positive for *terD* (Table 1) compared to 28 of 40 control organisms (70.0%; Table 2; $P < 0.001$). The K_2TeO_3 geometric mean MIC for STEC strains was 91.92 ± 1.02 mg/L (Table 1), which was not significantly different from that of the control organisms (19.82 ± 3.86 mg/L; $P = 0.375$; Table 2). The K_2TeO_3 geometric mean MIC for *terD*+ STEC strains was significantly higher than that for *terD*− STEC ($P < 0.0001$; Table 3). In contrast, the K_2TeO_3 geometric mean MIC for *terD*+ control organisms was not significantly different from that of *terD*− control organisms ($P = 0.6476$; Table 3). STEC isolates included in the analysis had insignificant symmetry of disagreement between the two testing methods by Bowker's test (0.3173); hence, they were subjected to Cohen's kappa coefficient (κ) analysis. The κ for agreement between presence or absence of *terD* and a resistant or susceptible phenotype, respectively, was 0.881 (CI 0.626–1.11, SE 0.11) indicating a very good level of agreement (Table 3). Resistance to K_2TeO_3 (MIC ≥ 25 mg/L) was exhibited by 65/66 *terD*+ (98.5%) and 0/4 *terD*− (0%) STEC strains (Table 3). Control organisms had insignificant symmetry of disagreement, with a Bowker's test score of 0.144. However, the κ for agreement between presence or absence of *terD* and a resistant or susceptible phenotype, respectively, was − 0.173 (CI − 0.455 to 0.108, SE 0.14) indicating no effective level of agreement. K_2TeO_3 resistance was exhibited by 12/28 *terD*+ control organisms (42.9%) in comparison to 8/12 *terD*− strains (66.7%; Table 3). A significant relationship was demonstrated

between presence of the gene for intimin (*eae*) and K_2TeO_3 resistance in STEC strains with a Pearson's χ^2 test of 5.7 ($P = 0.017$).

The prevalence of *terD* among adulterant STEC serogroups was 7 of 10 in O103 strains, whereas it was ≥ 9 of 10 in all other serogroups. The K_2TeO_3 geometric mean MIC varied among STEC serogroups, with a rank order from highest to lowest of O111 > O26 > O145 > O157 > O 103 > O121 = O45. Among individual strains, serogroups O26 and O111 consistently had a K_2TeO_3 MIC ≥ 100 mg/L, while 70% (7/10) and 80% (8/10) of serogroup O103 and O121 strains, respectively, had K_2TeO_3 MIC < 100 mg/L (Table 1). Serogroup O45 and O121 strains had the lowest mean K_2TeO_3 MIC (43.52 and 45.06 mg/L, respectively) while O111 strains had the highest (214.36 mg/L). Significant differences using ANOVA followed by Tukey–Kramer HSD on geometric mean MIC values were present between the O111 and O121 serogroups ($P < 0.0001$), O111 and O45 serogroups ($P = 0.0012$), O26 and O121 serogroups ($P = 0.0044$), O145 and O121 serogroups ($P = 0.0080$), O111 and O157 ($P = 0.0095$), and O26 and O45 serogroups ($P = 0.0437$), respectively. The MIC_{90} of serogroups O26, O103, O111, and O145 were 400 mg/L, whereas that of O45 was 200 mg/L and O121 was 100 mg/L.

Discussion

K_2TeO_3 has been used for over 100 years as a selective agent in media for the isolation of specific bacterial pathogens, e.g., *Corynebacterium diphtheriae, Staphylococcus aureus, Vibrio cholerae, Shigella* spp., and more recently, O157 STEC [16, 22]. In addition, K_2TeO_3 has been used to select for non-O157 STEC, but great variations in STEC tellurite susceptibilities exist among these organisms [12]. This variability in K_2TeO_3 resistance, coupled with a lack of unique biochemical markers, has hampered development of highly effective selective media for non-O157 STEC [4, 12, 20], prompting the need for more research and the study conducted herein.

The results of this study were consistent with those of Orth et al. [13] in that all O26, O111, O145, and O157 STEC strains tested were *terD*+ and there was a significant relationship between the presence of *eae* and K_2TeO_3 resistance. The results differed in that only seven of 10 O103 STEC strains in the present study (70%), in contrast to

two of two (100%) in the previous study [13], were *terD*+. The results of the present study extend the literature on K_2TeO_3 resistance and *terD* prevalence in USDA-FSIS-regulated STEC, and in particular O45 [3, 4, 6, 9, 10, 12, 13, 15, 17, 22]. The study by Orth et al. [13] included no O45 or O121 STEC strains. The study by Kerangart et al. [12] included only one O45 STEC strain, which was *terD*+, but did include 8–10 strains of each of the other serogroups. In general, our results parallel those of Kerangart et al. [12]; however, one difference was our finding that the geometric mean K_2TeO_3 MIC of O45 STEC was significantly lower than that of O26 and O111 STEC. Another was that the geometric mean K_2TeO_3 MIC of O157 STEC ranked in the middle among USDAFSIS- regulated STEC serogroups, whereas based on our analysis of LB broth MIC data presented in the supplementary data file of the study by Kerangart et al. [12] it was the lowest. Two of 12 O157:H7 STEC strains they tested were *terD*−, whereas all 10 O157:H7 STEC strains in our study were *terD*+.

The K_2TeO_3 MIC results of Orth et al. [13] were based on agar (LB and CT-SMAC), whereas Kerangart et al. [12] generated MIC data with both (LB) agar and (LB) broth, and they noted that agar yields values about one log lower than that of broth. However, Taylor et al. [17] determined the K_2TeO_3 MICs on a set of O157:H7 STEC strains on solid media and recorded values as high as 1024 μg/mL. Fukushima et al. [6] conducted a similar test of K_2TeO_3 MIC on solid media except they included non-O157 STEC as well. Although broth and agar MICs differ, our study confirmed the findings of Fukushima et al. [6] as K_2TeO_3 MIC varied by strain and serogroup, and expanded on it by including serogroup O45.

The fact that the Cohen's κ coefficient indicated very good agreement between presence or absence of *terD* and a resistant or susceptible phenotype in STEC strains suggests that K_2TeO_3 resistance in these organisms is a function of the *ter* gene cluster, which supports the results of previous studies [12, 13, 17]. The lack of resistance to K_2TeO_3 in over half of the *terD*+ control organisms suggests these strains lacked a necessary component of the *ter* gene cluster or lacked another component needed for functional K_2TeO_3 resistance. In addition, the presence of resistance in two-thirds of the *terD*− control organisms suggests that another of the at least five known mechanisms of K_2TeO_3 resistance was involved [16].

Conclusions

The prevalence of *terD* was significantly higher in STEC than in non-STEC control strains. The level of agreement between *terD* PCR and K_2TeO_3 minimum inhibitory concentration (MIC) was very good in STEC strains as determined by a Cohen's κ coefficient analysis, supporting and extending the results of previous studies suggesting that the genetic basis for K_2TeO_3 resistance in STEC is due to the *ter* gene cluster. In contrast, there was a significant disagreement between *terD* PCR and K_2TeO_3 MIC in non-STEC control organisms. The presence of resistance in nearly half of the *terD*– control organisms suggests that another mechanism of K_2TeO_3 resistance was involved. The seven USDA-FSIS adulterant serogroups varied in prevalence of *terD* and levels of K_2TeO_3 resistance. Among STEC serogroups, the prevalence of *terD* was lowest in O103 STEC strains (70%), whereas the prevalence in all other serogroups was ≥ 90% (9 of 10). These results confirm previous studies showing the significantly higher prevalence of the *ter* gene cluster in STEC strains, and the relationship between presence of these genes and K_2TeO_3 resistance in STEC and especially intimin (*eae*)-positive STEC, in contrast to non-STEC organisms. These results expand on the literature by the finding that O45 and O121 STEC, although frequently *terD* positive, on average have significantly lower levels of $K2TeO_3$ resistance than USDA-regulated serogroups O26, O111, and O145.

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Conflict of interest — Dr. Loy has served as a consultant for, and thus has disclosed a significant financial interest in Harrisvaccines. In accordance with its Conflict of Interest policy, the University of Nebraska–Lincoln's Conflict of Interest in Research Committee has determined that this must be disclosed.

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