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Evaluation of eight agar media for the isolation of shiga toxin—Producing *Escherichia coli*

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ABSTRACT

The growth characteristics of 96 shiga toxin-producing *Escherichia coli* (STEC) strains representing 36 different O-types (including priority O types O26, O45, O103, O111, O121, O145 and O157) on commercial and in-house agar media were studied. The ability of the strains to grow on agar media with varying selective supplement formulations was evaluated using MacConkey Agar (MAC); Rainbow® Agar O157 (RBA); Rainbow® Agar O157 with manufacturer-recommended selective supplements (RBA-NT); Rainbow® Agar O157 with USDA-recommended selective supplements (RBA-USDA); CHROMagar STEC™ (CH STEC); Tryptone Bile agar containing cefixime and tellurite (TBA-CT); Tryptone Bile agar containing cefixime, tellurite, eosin and methylene blue (TBA-EM); and VTEC agar. All of the strains were able to grow on MAC, RBA and VTEC agar, whereas a number of strains (including some non-O157 priority O types) were unable to grow on the highly selective media CH STEC, RBA-NT, RBA-USDA, TBA-EM and TBA-CT. Only RBA-NT and CH STEC exhibited significant inhibition of background flora from ground beef enrichment. Significant inhibition of background flora from beef trim enrichment was observed with RBA-NT, RBA-USDA, CH STEC, TBA-EM and VTEC agar. With exception of *E. coli* O157, several different colony morphologies were observed on the differential plating media among strains of the same O type, indicating that this colony morphology is not a reliable means of identifying target STEC. These results suggest that an approach to maximize the recovery of target STEC from beef enrichment cultures is dual plating on lesser (RBA, MAC, VTEC agar) and more highly (RBA-NT, CH STEC) selective agars.

1. Introduction

Shiga toxin-producing *Escherichia coli* (STEC) (also known as verotoxin producing *E. coli* or enterohemorrhagic *E. coli*) are the enteric *E. coli* pathotypes of the greatest public health significance in the industrialized world due to their low infectious dose, potentially severe patient outcomes and limited treatment options (Melton-Celsa et al., 2012).

Development of methods for the detection and isolation of STEC has focused on *E. coli* O157:H7 and its nonmotile variant (*E. coli* O157) as this serotype has been responsible for a number of major foodborne outbreaks and is the cause of the majority of reported cases in some regions, including Canada and the USA (CDC, 2012; PHAC, 2010). Other serotypes of STEC (non-O157 STEC) are estimated to account for 50–60% of cases of STEC illness in Canada and the USA (CDC, 2012; Thompson et al., 2005; Chui et al., 2011). The serotypes of STEC that predominate as a cause of human illness vary between geographic regions (EFSA, 2007; Johnson et al., 2006).

The diversity of STEC serotypes causing disease is high, at least 82 different O-types of STEC have been reported as clinical isolates from three or more outbreaks or unlinked sporadic cases (Bettelheim, 2007). This situation is probably a consequence of the mobility of the verotoxin genes, which are encoded by a lysogenic phage (Kaper et al., 2004). Thus, there is a high probability of novel STEC pathogens emerging, such as the verotoxin-positive enteroaggregative *E. coli* O104:H4 responsible for the 2011 outbreak in Germany (Beutin and Martin, 2012).

Isolation of STEC from enrichment broth cultures of foods or other sample types requires an agar medium for the isolated growth of individual colonies. Such a medium should support the growth of the target organism and ideally have selective and differential characteristics to support the rapid identification of pathogen colonies in the presence of other microbial flora. There is a variety of commercially available media for *E. coli* O157 which meets these requirements (Heuvelink, 2012). These media are commonly based on differential characteristics typical to *E. coli* O157 but unusual in other *E. coli*, notably the absence of sorbitol fermentation and β-D-glucuronidase activity. Selectivity is achieved by the relatively high resistance of *E. coli* O157 to some antimicrobials, such as novobiocin and tellurite, compared to other *E. coli*. These differential and selective characteristics are not shared by other STEC (Gill et al., 2012) and to date the only characteristic known to

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Table 1
Characteristics of STEC strains and their percentage recovery on selective agar media compared to Brain Heart Infusion agar.[a]

Serotype[a]	Strain ID	Isolation	*stx1*	*stx2*	*eae*	*hlyA*	MAC	RBA	RBA-NT	RBA-USDA	CH STEC	TBA-CT	TBA-EM	VTEC
O26:NM	11-6009	Human	+	−	+	−	106%	124%	133%	51%	104%	102%	114%	120%
O26:H11	05-7321	Human	+	−	+	+	119%	94%	121%	188%	106%	91%	104%	121%
O26:H11	05-6544	Human	+	−	+	+	123%	157%	20%	117%	<0.1%%	87%	97%	150%
O26:H11	03-2816	Human	+	−	+	−	133%	123%	111%	52%	119%	101%	103%	188%
O26:H11	01-5870	Human	+	−	+	+	118%	95%	103%	47%	62%	90%	72%	108%
O26:H11	02-6737	Human	+	+	+	+	33%	67%	52%	95%	52%	57%	95%	81%
O26:H11	99-4610	Human	+	−	+	+	133%	100%	108%	108%	62%	67%	100%	100%
O26:H21	11-5130	Human	+	−	+	−	85%	72%	83%	71%	70%	83%	98%	102%
O26:H21	11-5593	Human	+	+	−	+	95%	158%	NG	NG	NG	71%	58%	105%
O26:H21	11-5805	Human	+	−	+	+	113%	103%	105%	175%	103%	89%	89%	92%
O45:H2	04-2445	Human	+	−	+	+	135%	93%	71%	55%	68%	132%	103%	132%
O45:H2	05-6545	Human	+	−	+	+	81%	73%	49%	40%	7%	76%	90%	71%
O45:H2	85-X-40c R3	Cattle	+	−	+	+	102%	131%	63%	35%	19%	95%	126%	84%
O45:H2	3267-95	Human	+	−	+	+	116%	113%	60%	22%	18%	68%	84%	108%
O45:H2	3285-96	Human	+	−	+	+	72%	126%	126%	27%	65%	81%	102%	63%
O45:H2	89-39	Human	+	−	+	+	94%	111%	52%	33%	19%	20%	98%	127%
O103:H2	99-2076	Human	+	−	+	+	53%	41%	NG	NG	NG	NG	NG	94%
O103:H2	06-0434	Human	+	−	+	+	86%	110%	100%	96%	45%	103%	83%	117%
O103:H2	04-2446	Human	+	−	+	+	79%	74%	77%	130%	32%	81%	121%	145%
O103:H2	95-266	Human	+	−	+	+	167%	189%	NG	NG	NG	189%	87%	122%
O103:H2	09-5066	Human	+	−	+	+	81%	104%	13%	23%	<0.1%	71%	85%	81%
O103:H2	11-5806	Human	+	−	+	+	110%	95%	46%	79%	46%	115%	115%	103%
O103:H11	04-3973	Human	+	−	+	+	89%	85%	75%	100%	69%	109%	106%	115%
O103:H21	11-4211	Human	+	−	+	+	81%	96%	56%	100%	30%	107%	93%	93%
O103:H21	11-5595	Human	+	−	+	+	126%	92%	97%	100%	92%	113%	139%	105%
O103:H25	03-2444	Human	+	−	+	+	67%	83%	3%	25%	<0.1%	37%	45%	92%
O111:NM	05-4161	Human	+	+	+	−	126%	174%	53%	63%	42%	111%	153%	137%
O111:NM	98-8338	Human	+	−	+	+	109%	98%	NG	NG	NG	<0.1%	<0.1%	89%
O111:NM	CFS3	Human	+	+	+	+	96%	82%	7%	96%	20%	51%	65%	76%
O111:NM	00-4748	Human	−	+	+	−	2%	8%	NG	<0.1%	NG	<0.1%	<0.1%	33%
O111:NM	00-4440	Human	+	−	+	+	34%	68%	28%	60%	6%	38%	49%	36%
O111:NM	11-5592	Human	+	−	+	+	82%	158%	85%	103%	35%	127%	61%	88%
O111:NM	03-3991	Human	+	−	+	+	100%	71%	32%	29%	26%	65%	65%	68%
O111:H8	3331-00	Unknown	+	+	+	+	100%	100%	30%	39%	9%	52%	74%	113%
O111:H8	3413-07	Unknown	+	+	+	+	103%	132%	NG	67%	NG	<0.1%	132%	156%
O111:H11	OLC-455	Unknown	+	−	+	+	87%	93%	50%	60%	27%	53%	83%	73%
O121:NM	03-4064	Human	−	+	+	+	179%	53%	<0.1%	94%	42%	128%	137%	133%
O121:H1	11-5594	Human	−	+	+	+	214%	145%	73%	111%	73%	159%	168%	145%
O121:H1	11-5597	Human	−	+	+	+	75%	100%	49%	67%	31%	82%	88%	88%
O121:H10	96-0120	Unknown	−	+	−	−	90%	115%	NG	<0.1%	NG	<0.1%	100%	85%
O121:H19	11-2925	Human	−	+	+	+	89%	88%	85%	115%	84%	68%	78%	91%
O121:H19	11-3925	Human	−	+	+	+	111%	100%	32%	6%	11%	61%	70%	74%
O121:H19	11-4440	Human	−	+	+	−	113%	105%	118%	71%	125%	95%	105%	20%
O121:H19	03-2832	Human	−	+	+	+	108%	119%	33%	77%	8%	64%	106%	81%
O121:H19	00-5288	Human	−	+	+	+	123%	74%	51%	18%	4%	118%	111%	102%
O121:H19	03-2642	Human	−	+	+	+	118%	97%	21%	80%	5%	33%	92%	105%
O145:NM	04-1449	Human	+	−	+	+	116%	100%	68%	67%	48%	55%	52%	106%
O145:NM	04-7099	Human	+	−	+	+	103%	125%	67%	49%	47%	122%	94%	106%
O145:NM	03-6430	Human	+	−	+	+	136%	130%	70%	52%	70%	115%	130%	121%
O145:NM	03-4699	Human	+	−	+	+	111%	100%	29%	43%	20%	95%	78%	93%
O145:NM	2454-01	Human	+	−	+	+	98%	115%	63%	82%	13%	67%	71%	81%
O145:NM	VT113-5	Human	−	+	+	+	36%	62%	53%	32%	6%	53%	54%	7%
O145:H2	A9619.C2	Human	+	−	−	−	33%	58%	NG	NG	NG	NG	NG	38%
O145:H2	75-83	Human	−	+	+	+	103%	69%	53%	42%	NG	56%	83%	94%
O145:H25	2769	Human	−	+	+	+	117%	117%	60%	39%	33%	81%	90%	88%
O157:H7	ATCC 35150	Human	+	+	+	+	83%	72%	29%	34%	14%	108%	100%	75%
O157:H7	1011-84	Human	+	+	+	+	100%	103%	18%	51%	9%	36%	45%	115%
O157:H7	HCO 59	Human	+	+	+	+	104%	85%	30%	35%	11%	67%	81%	107%
O157:H7	11-1024	Human	−	+	+	+	143%	152%	19%	78%	5%	81%	129%	86%
O157:H7	11-1865	Human	−	+	+	+	81%	88%	38%	75%	12%	88%	62%	112%
O157:H7	EDL933	Human	+	+	+	+	95%	18%	108%	29%	8%	60%	80%	90%
O157:H7	Sakai	Human	+	+	+	+	112%	160%	36%	21%	17%	112%	93%	105%
O157:NM	ER63-94	Human	+	+	+	+	87%	67%	29%	78%	15%	98%	79%	71%
O157:NM	E32511	Human	−	+	+	+	122%	83%	65%	53%	9%	78%	96%	122%
O157:NM	87-1215	Human	+	+	+	−	114%	129%	114%	86%	57%	171%	86%	57%
O1:H20	91-0812	Unknown	+	−	−	+	119%	113%	NG	19%	<0.1%	59%	120%	107%
O5:NM	03-2682	Human	+	−	+	+	97%	109%	91%	92%	51%	74%	87%	98%
O6:H34	03-5166	Human	−	+	−	−	123%	94%	NG	NG	NG	60%	125%	114%
O7:H4	92-0249	Unknown	−	+	−	−	170%	128%	NG	<0.1%	NG	87%	113%	143%
O8:H19	09-1764	Unknown	+	+	−	+	109%	123%	<0.1%	NG	<0.1%	85%	88%	106%
O46:H38	97-0757	Human	+	+	−	+	83%	81%	32%	47%	23%	73%	87%	108%
O55:H7	05-0376	Human	+	−	+	−	127%	107%	NG	NG	NG	83%	82%	110%
O69:H11	11-5596	Human	+	−	+	+	143%	129%	114%	83%	129%	57%	86%	71%
O76:H19	09-0523	Unknown	+	+	−	+	100%	120%	NG	NG	NG	117%	92%	80%

(continued on next page)