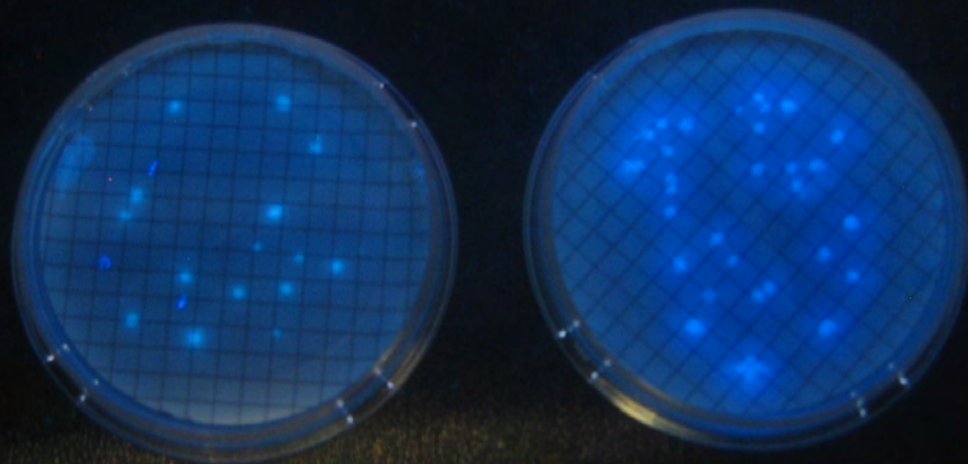![USGS logo]

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Prepared in cooperation with California State Water Resources Control Board

Identification of Bacteria in Groundwater Used for Domestic Supply in the Southeast San Joaquin Valley, California, 2014

Scientific Investigations Report 2021–5030

U.S. Department of the Interior
U.S. Geological Survey

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Conversion Factors

International System of Units to U.S. customary units

Multiply	By	To obtain
Length		
nanometer (nm)	0.00000003947	inch (in.)
micrometer (µm)	0.00003937	inch (in.)
meter (m)	3.281	foot (ft)
kilometer (km)	0.6214	mile (mi)
Area		
square meter (m^2)	10.76	square foot (ft^2)
square kilometer (km^2)	0.3861	square mile (mi^2)
Volume		
liter (L)	1.057	quart (qt)
cubic meter (m^3)	264.2	gallon (gal)
Flow rate		
cubic meter per day (m^3/d)	35.31	cubic foot per day (ft^3/d)
Mass		
gram (g)	0.03527	ounce, avoirdupois (oz)
kilogram (kg)	2.205	pound avoirdupois (lb)
Pressure		
kilopascal (kPa)	0.1450	pound per square inch (lb/ft^2)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:

$$°F = (1.8 × °C) + 32.$$

Datum

Vertical coordinate information is referenced to the North American Vertical Datum of 1988 (NAVD 88).

Horizontal coordinate information is referenced to the North American Datum of 1983 (NAD 83).

Abbreviations

BLAST	basic local alignment search tool
CDC	Centers for Disease Control and Prevention
CFU	colony forming unit
DNA	deoxyribonucleic acid
EPA	U.S. Environmental Protection Agency
FIB	fecal indicator bacteria
GAMA-PBP	Groundwater Ambient Monitoring and Assessment Program Priority Basin Project
MCL	maximum contaminant level
PAH	polycyclic aromatic hydrocarbon
PCR	polymerase chain reaction
rDNA	ribosomal deoxyribonucleic acid
rRNA	ribosomal ribonucleic acid
Taq polymerase	*Thermus aquaticus* polymerase
TC	total coliforms
USGS	U.S. Geological Survey

Identification of Bacteria in Groundwater Used for Domestic Supply in the Southeast San Joaquin Valley, California, 2014

By Carmen A. Burton[1] and Christine J. Lawrence[2]

Abstract

Groundwater is an important source of drinking water in California. Water-borne diseases caused by microbial contamination are a growing concern. The MI test, a membrane filtration method for the chromogenic/fluorogenic detection of total coliforms and *Escherichia coli*, was used for samples collected January to April 2014 from 42 domestic wells in the southeastern San Joaquin Valley. The wells were sampled as part of the Groundwater Ambient Monitoring and Assessment Program Priority Basin Project (GAMA-PBP), a cooperative study between the U.S. Geological Survey and the California State Water Resources Control Board. Polymerase chain reaction analysis and sequencing of deoxyribonucleic acid (DNA) were used for 34 target and nontarget colonies that grew on the MI media from samples collected from 13 of the domestic wells to identify what genera of bacteria could exist in groundwater used by domestic wells. Gene sequences obtained using the Sanger method were entered into the basic local alignment search tool (BLAST) database, and 17 genera of bacteria were identified. Of these, 13 genera contain species that are human pathogens or opportunistic human pathogens. All the genera that include human pathogens are naturally present in soil, plants, or water; one of the pathogens also can be found in fecal matter. Six of the human pathogens were from non-target colony growth on the MI media. Target and non-target microbial growth on MI media are indicators of the possible presence of pathogenic bacteria even if the bacteria naturally are from soil rather than from a fecal source.

[1]U.S. Geological Survey

[2]Formerly with the U.S. Geological Survey

Introduction

Diseases can be spread by waterborne microbial pathogens in groundwater. Most waterborne microbial pathogens are derived from human or animal waste; however, evidence exists that many microorganisms can survive in viable but nonculturable forms in non-fecal conditions (Levin and others, 2002). For the years 2013–14, the Centers for Disease Control and Prevention (CDC) listed 42 reported outbreaks associated with drinking water (Benedict and others, 2017). Of the 42 outbreaks, 9 were caused by pathogens in groundwater. To test for each pathogen is difficult, prohibitively costly, and risks pathogenic exposure to the person doing the test. Fecal indicator bacteria (FIB), such as total coliforms (TCs) and enterococci, are commonly used to indicate the possible presence of waterborne disease-causing organisms of fecal origin. Fecal indicator bacteria were chosen as indicators of potential microbial contamination in the U.S. Environmental Protection Agency's (EPA) "total coliform rule and ground water rule" because they generally are not pathogenic, are easy to culture, and can be fecal in origin (U.S. Environmental Protection Agency, 2008, 2020b).

Coliforms inhabit the intestines of warm-blooded animals and are found in feces. Coliforms also are present in soil, on vegetation, and in water (Foppen and Schijven, 2006; Brennan and others, 2010; Krentz, 2012). Studies show that *Escherichia coli* (*E. coli*), one species of coliform always found in feces, and enterococci, another group of bacteria that are mostly of fecal origin, could be better indicators of fecal contamination than TCs (U.S. Environmental Protection Agency, 1986).

About 60 percent of the water used for public and domestic drinking-water supply in California is groundwater (Dieter and others, 2018). Public-supply wells are routinely tested for TCs and *E. coli* as part of regulatory compliance monitoring, and corrective actions are taken if the maximum contaminant levels (MCLs) are exceeded (U.S. Environmental Protection Agency, 2008; California State Water Resources Control Board, 2019). In contrast, the State of California and the EPA do not regulate water quality in privately owned domestic wells (California State Water Resources Control Board, 2015; U.S. Environmental Protection Agency, 2020a). In a study by the U.S. Geological Survey (USGS) of domestic wells from 1991 to 2004, 34 percent of wells tested positive for TCs and 8 percent tested positive for *E. coli* (DeSimone and others, 2009). Testing of domestic wells in six California counties by the California State Water Resources Control Board found 14 to 33 percent of the wells tested positive for TCs, with the highest frequency in Tulare County in the southeast San Joaquin Valley (California State Water Resources Control Board, 2018). No information is available concerning the genera of bacteria causing these TC detections in the California domestic wells, however. Knowledge about the genera gives information about the possible origin of the bacteria, which informs what type of action might be needed to protect the well or the consumers of the water from the well from microbial contamination. In addition, knowledge of the genera could be useful for assessing the selectivity and specificity of commonly used TC tests for indicating the presence of potentially pathogenic bacteria.

The EPA has approved several analytical methods for monitoring FIBs including traditional membrane filtration methods and defined substrate technology (Covert and others, 1989; Brenner and others, 1996; U.S. Environmental Protection Agency, 2002, 2006, 2008; Buckalew and others, 2006; Oldstadt and others, 2007; Zhang and others, 2015). Polymerase chain reaction (PCR) analysis is widely used to identify bacteria. In particular, the 16S ribosomal ribonucleic acid (rRNA) gene is a well-studied universal gene in bacteria and is a standard method for the identification of species (Barry and others, 1991; Jensen and others, 1993; Gürtler and Stanisich, 1996; Clermont and others, 2000; Ryu and others, 2013). The membrane filtration methods result in bacterial colonies that can be used for PCR analysis for identification of bacteria in the water samples that the defined substrate methods do not. Two common membrane filtration methods use MI medium (Brenner and others, 1993; U.S. Environmental Protection Agency, 2002) and mEI medium (U.S. Environmental Protection Agency, 2006).

The USGS has an ongoing statewide assessment of water quality in groundwater resources used for drinking-water supplies for the California State Water Resources Control Board's Groundwater Ambient Monitoring and Assessment Program Priority Basin Project (GAMA-PBP). The FIB samples were collected as part of a GAMA-PBP study of domestic wells in the southeastern part of the San Joaquin Valley from January to April 2014 (fig. 1). As the FIB tests used in this study are not genera- or species-specific, the objective of this study was to identify the genera of bacteria cultured by these FIB methods from samples collected at domestic wells. The purposes of this report are to (1) identify some of the genera of bacteria that can be present in domestic wells in the San Joaquin Valley, (2) examine potential pathogenicity of bacteria cultured on MI media, and (3) present results of a literature review describing the general sources of bacteria identified by FIB methods.
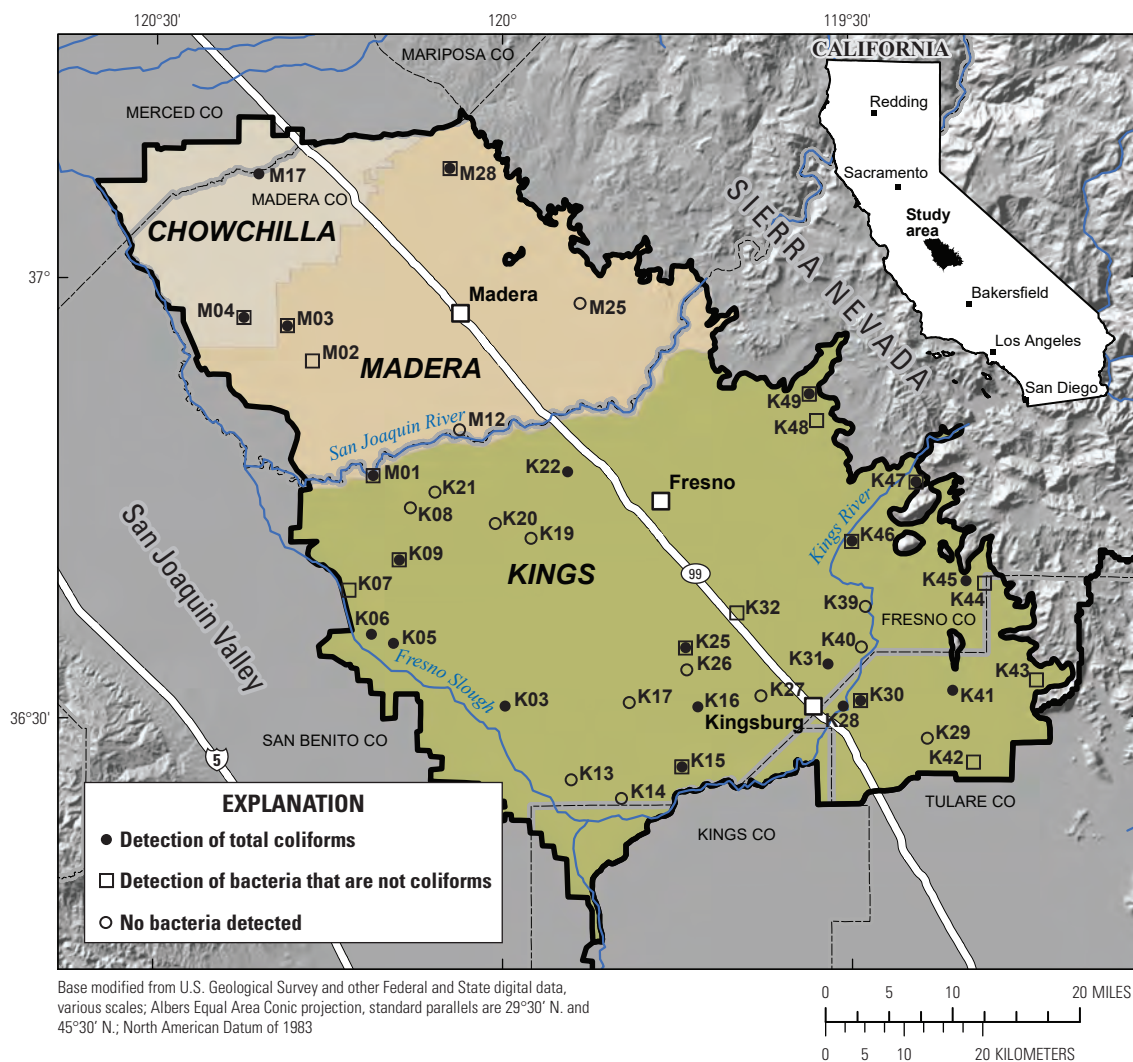
Figure 1. Location of the Chowchilla, Madera, and Kings groundwater subbasins of the San Joaquin Valley, California, well sites, and type of microbial detections for samples collected from January to April 2014.

Methods

Sampling Locations

This study was part of the GAMA-PBP assessment of domestic wells in the Chowchilla, Madera, and Kings groundwater subbasins of the San Joaquin Valley (fig. 1; Shelton and Fram, 2017). This region was selected for study because many of the households rely on domestic wells as their sole source of drinking-water supply (Johnson and Belitz, 2015). The aquifer system used by domestic wells in this region of the San Joaquin Valley primarily consists of Quaternary unconsolidated alluvial fan and fluvial gravel, sand, silt, and clay sediments interbedded with lesser amounts of lacustrine deposits (Davis and others, 1959; Faunt, 2009;

California Department of Water Resources, 2003, 2008a, b). The sediments were derived from the weathering of the mostly granitic rocks of the Sierra Nevada. More than 60 percent of the wells sampled were in areas where 40 percent or more of the aquifer sediments consisted of coarse-grained sediments, and recharge rates, as measured by downward vertical flux, typically ranged from 1,600 to more than 7,600 cubic meters per day (Faunt, 2009). Groundwater generally flows westerly from the Sierra Nevada. Samples from 42 wells of the 77 wells sampled for the GAMA-PBP assessment were analyzed to identify the genera of bacteria that can exist in the well water. Well depths for the 42 wells ranged from 30 to 104 meters (median 55 meters), and depth to water was 9 to 67 meters (median 28 meters; Shelton and Fram, 2017). Samples were identified by a shortened form of the GAMA-PBP identification number given in Shelton and Fram (2017).

Sample Collection

Groundwater samples were collected using protocols described in USGS National Field Manual (U.S. Geological Survey, variously dated) from January to April 2014 (Shelton and Fram, 2017). Domestic wells were purged, removing one or more well volumes of standing water depending on estimated water use of the domestic wells in the previous 24 hours, until at least three casing volumes of water were removed per standard USGS procedures. Purging continued until field measurements of pH, temperature, dissolved oxygen, and specific conductance stabilized (U.S. Geological Survey, variously dated). Prior to the collection of microbial samples, the sample point was disinfected by spraying it inside and out with ethanol, allowing it to dry, and then rinsing it with sterile deionized water. Water samples were collected in sterile polypropylene bottles from points prior to where the water passes through any holding tanks or chemical treatment systems. Samples were shipped overnight on ice to the USGS California Water Science Center in San Diego, Calif., for analysis. Duplicate samples were collected at six sites (14 percent of the sites).

Analysis of Total Coliforms, *Escherichia coli*, and Enterococci

The presence of TCs and *E. coli* was determined by USEPA Method 1604, a membrane filtration method on MI medium (U.S. Environmental Protection Agency, 2002). The presence of enterococci was determined by USEPA Method 1600, a membrane filtration method on mEI medium (U.S. Environmental Protection Agency, 2006). The MI medium includes a fluorogen that reacts with the enzyme β-galactosidase found in TCs and a chromogen that reacts with the enzyme β-glucuronidase found in *E. coli*. A cephalosporin antibiotic, cefsulodin, is added to the MI medium to inhibit non-target growth. The mEI medium includes the chromogen indoxyl β-D-glucoside, which interacts with β-glucosidase-positive enterococci. Samples were analyzed within 24–30 hours of collection. All equipment was sterilized by autoclave at 121 degrees Celsius (°C) and 103 kilopascals for at least 15 minutes. Sterile phosphate buffer was obtained from Hardy Diagnostics (Santa Maria, Calif.). The MI and mEI media were obtained from the USGS Ohio Microbiology Lab, Columbus, Ohio. Positive and negative control cultures were obtained from IDEXX Laboratories (Westbrook, Maine).

Microbial samples were processed using protocols described in the USGS National Field Manual (Myers and others, 2014). Aliquots of 250 milliliters (mL) of sample were vacuum filtered onto 0.45 micrometer (µm) gridded membranes, and the membrane was placed on plated media. An aliquot of 250 mL, instead of the usual 100 mL, was filtered to increase the chances of obtaining bacterial growth. Two aliquots of sample were filtered for each type of media. Samples on MI media were incubated at 35 °C for 22–26 hours, and samples on mEI media were incubated at 41 °C for 22–26 hours. After incubation, the bacteria colonies on the plates were counted. Any plates showing growth were stored at 4 °C and retained for sequencing analysis. Plates were stored for up to 4 days before further analysis.

Quality control consisted of positive and negative controls cultured on each lot of medium used; field, filter, and procedure blanks; and field duplicates. Positive control bacteria used for MI medium were *E. coli* and *Klebsiella pneumoniae*, and the negative control was *Pseudomonas aeruginosa*. The positive control for mEI medium was *Enterococcus faecalis*, and the negative controls were *E. coli* and *Streptococcus bovis*. A total of eight sets of positive and negative control samples were analyzed, one at the start of each lot of media used and one at the end of each lot of media used during the study. In all cases, the positive controls showed colony growth, and the negative controls did not, indicating each lot of media for the MI and mEI methods performed as required.

Field blanks were collected from six sites using sterile phosphate buffer and processed using the same procedures as for environmental samples. Field blanks were used to assess contamination introduced during the collection process. Filter blanks were processed before each sample was plated. Procedure blanks were processed between sample aliquots plated on MI medium and on mEI medium. For filter and procedure blanks, 50 mL of sterile phosphate buffer was filtered through a 0.45-µm gridded membrane filter. The membrane filter was plated on MI or mEI media. The filter blanks were used to assess contamination introduced in the laboratory during sample preparation activities. The procedure blank was used to test for completeness and sterility of the filtering process from one aliquot to the next. All field blanks, filter blanks, and procedure blanks showed no colony growth, which indicated no contamination by FIBs was introduced during collection and processing of the samples. Duplicates were collected from six sites. For one duplicate pair, TC was detected in the environmental sample but not the replicate; there were no detections of *E. coli* or enterococci for either sample of all six duplicate pairs; and three duplicate pairs yielded growth of non-target organisms (colonies that do not fluoresce blue) on MI media in the duplicate but not the environmental sample.

The definition of a coliform varies depending on the country or organization in charge of microbiological regulations and the method used. For MI media, the definition of a coliform is any bacteria that fluoresces blue-green or blue-white under longwave ultraviolet light (366 nanometers). A phenotypic definition is more specific and defines coliforms as rod-shaped, Gram-negative, non-spore forming bacteria that ferment lactose with production of acid and gas when incubated at 35–37 °C and reacts positively with ß-D-galactosidase. For simplicity, with a few exceptions, this report uses the MI definition of a coliform.

## Sequencing Analysis of the 16S rRNA Gene Regions

The universal 16S rRNA gene is a component of a prokaryotic ribosome, a complex of 16S rRNA and ribonucleoproteins. The parts of the rRNA that carry out essential functions are highly conserved across nearly all bacterial species (Jensen and others, 1993; Gürtler and Stanisich, 1996). The structural parts of the rRNA are largely variant, however, and are referred to as hypervariable regions. The sequence of ribosomal deoxyribonucleic acid (rDNA) in the hypervariable regions of bacteria is used for phylogenetic analysis and genera and species identification (Barry and others, 1991; Jensen and others, 1993; Gürtler and Stanisich, 1996).

Sequencing of a part of the hypervariable region of rDNA followed the procedures modified from Barry and others (1991). In general, the universal primers are used to flank a sequence of the 16S hypervariable region of rDNA, then PCR is used to amplify this region, and the region is sequenced and the genera and species containing that sequence are identified. Three sets of primers and two annealing temperatures were tested on cultures of *Klebsiella pneumoniae*, *Enterococcus faecalis*, and *E. coli* to determine which set of universal primers and temperature to use.

The forward universal primer 8F (5'-AGAGTTTGATCCTGGCTCAG-3') and the reverse universal primer 787R (5'-CGACTACCAGGGTATCTAAT-3') were selected to amplify a region of 16S rDNA that codes for a component of the 30S small subunit of prokaryotic ribosomes and includes the hypervariable regions that can provide a sequence to identify a genus or species of bacteria. A sample from a target colony or a non-target colony that grew on the MI media was taken by dipping a sterile pipette tip into the colony, and the cells were transferred into a PCR tube with 25 microliters (µL) of a PCR solution mix. The mix contained 5 µL of 5X Q-solution, 2.5 µL of tris-borate-EDTA 10X buffer, 1.25 µL of a 25 millimolar magnesium chloride solution, 1.25 µL of deoxynucleotide triphosphate (dNTP) solution, 0.5 µL of 10 micromolar solutions of each of the two primers, and 0.125 µL of *Taq* polymerase in deionized water. Amplifications used a Bio-Rad T100 Thermocycler and included a 3-minute denaturing step at 95 °C, followed by 30 cycles of 30 seconds at 94 °C, an annealing step at 55.2 °C for 45 seconds, followed by 1 minute at 72 °C, and a final extension of 10 minutes at 72 °C. Primers were ordered from Operon Lab, *Taq* polymerase from Fisher Scientific, and dNTP mix from Life Technologies. Phase-streaking of the selected colonies was not done to ensure that the colonies of interest were from a single bacteria cell before PCR analysis. It is therefore possible that cells from more than one species were added to the PCR mix.

The results of the PCR were analyzed with gel electrophoresis using Lonza FlashGel. If the PCR reaction was successful, a distinct band or smudge is seen around 700 base pairs (fig. 2). Samples for which the PCR products show a distinct band or smudge on the gel were purified with UltraClean PCR Clean-Up Kit from Mo Bio Laboratories. The purified PCR product was sequenced by the Sanger sequencing method (Sanger and Coulson, 1975; Sanger and others, 1977) by GeneWiz Laboratory, San Diego, Calif. The sequence was entered into the basic local alignment search tool (BLAST) database for species identification (National Center for Biotechnology Information, 2019). Along with the genus and species identification, the percentage of query coverage and identity were obtained. Query coverage is the percentage of the sequence length of the colony DNA that is aligned with DNA segments of the BLAST database sequence. Identity is the extent to which the two sequences have the same nucleotide at the same position in an alignment (Fassler and Cooper, 2011). The greater the query coverage or identity, the more likely that the correct genus or species is identified. Quality control consisted of testing the primers with cultures of *K. pneumoniae*, *E. coli*, *P. aeruginosa*, and *E. faecalis*. All the control bacteria were correctly identified. Information obtained from the literature was used to determine the most likely genus or species when the BLAST database produced multiple options for a given sequence. The DNA-sequence data are published in a companion USGS ScienceBase data release (Soldavini and others, 2021).
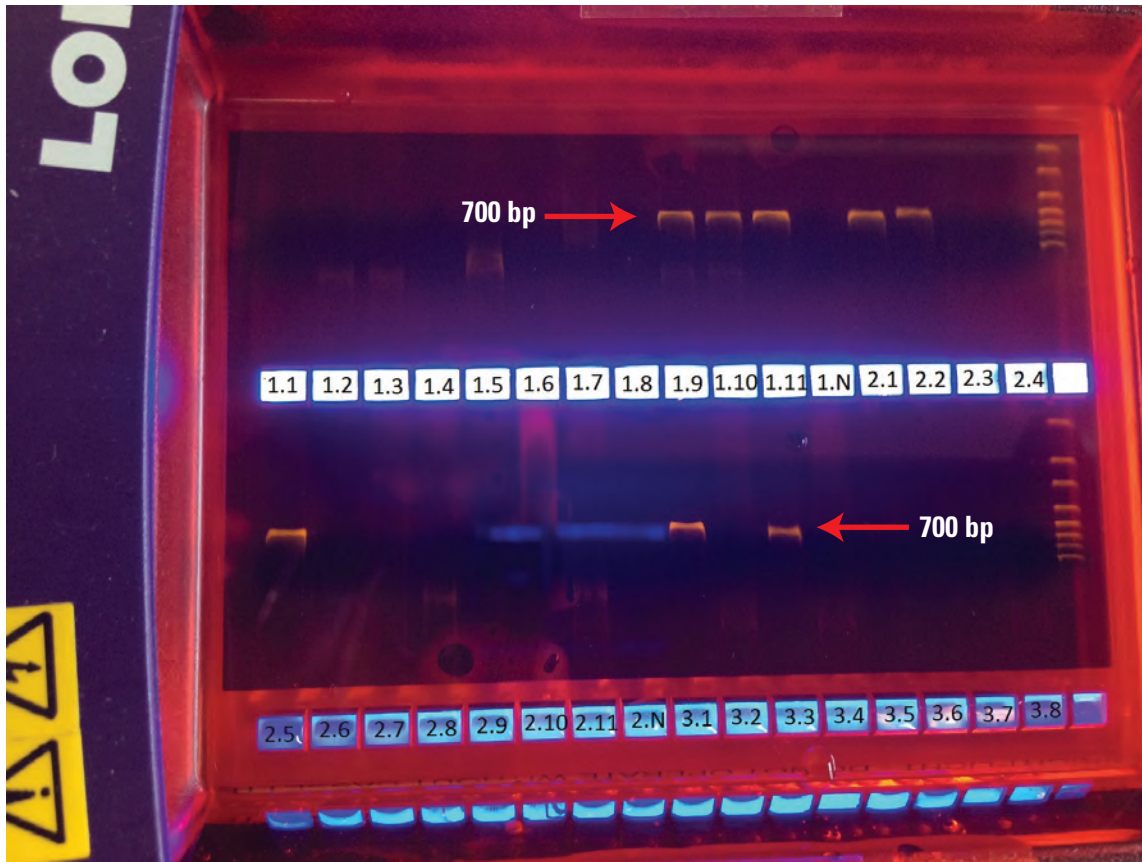
Figure 2.   Results of electrophoresis on a Lonza FlashGel cassette showing bands at 700 base pairs (bp) indicating successful polymerase chain reaction (PCR) amplification reactions.

Results

Samples from 27 of the 42 domestic groundwater wells had at least one colony forming unit (CFU) of either TCs, non-target growth, or both (table 1). *E. coli* and enterococci were not observed in any of the samples. Samples from 20 of the 42 wells had at least one CFU of TCs, and samples from 18 of the 42 wells had at least one CFU of non-target organisms. For samples with detections of TC, half also had non-target growth. For those samples without detections of TC, nearly one-third had non-target growth. The range of colonies counted per sample with growth (including duplicates) on the MI media was 1 to 144 (median = 4).

PCR was run on 106 of the 579 colonies that grew on the MI media during this study: 57 target colonies and 49 non-target colonies. Amplification of the section of DNA flanked by the primers was successful for 59 colonies based on the FlashGel electrophoresis and sent to GeneWiz for sequencing of the bases. Sequencing was successful for 34 of the 59 colonies (19 from TC colonies and 15 from non-target colonies). The gene sequences were entered into the BLAST database for identification, and the results are given in table 2. The number of bases in a sequence ranged

from 450 to 901 bases. Seventeen different genera were identified. The percentages of query coverage and identity that indicate the certainty of genus or species identification also are shown in table 2. The query coverage of the gene sequences ranged from 79 to 100 percent (median 100 percent, mean 98.3 percent), and the certainty of identity ranged from 87 to 100 percent (median 99 percent, mean 97.8 percent; table 2). Even when the percentages of query coverage or certainty of identity were high (95 percent or higher), five colonies had more than one potential genus identified (for example, K30 and K49 each had two potential genera). Information about habitat and source obtained from the literature (table 3) was used to determine which genus was most likely to be correct when the BLAST database produced multiple genera for a given sequence.

The MI test defines TCs as bacteria that produce blue-fluorescent colonies upon exposure to longwave ultraviolet light after culturing on MI medium. Four genera of bacteria, *Achromobacter*, *Chryseobacterium, Cupriavidus*, and *Pseudomonas*, were identified in both target and non-target colonies from samples collected from domestic wells, but not in the same well. The bacteria are not coliform bacteria and should not have been identified from target colonies.

Table 1.   List of domestic sites sampled for fecal indicator bacteria and number of colony-forming units (CFUs) per 250 milliliters of sample from cultures on MI and mEI media, southeastern San Joaquin Valley, January to April 2014.

[Well identification number is a shortened form of the GAMA-PBP identification number given in Shelton and Fram (2017). *Escherichia coli* and enterococci were not detected in any of the samples. **Abbreviations**: GAMA-PBP; Groundwater Ambient Monitoring and Assessment Priority Basin Project; USGS, U.S. Geological Survey; mm/dd/yyyy, month/day/year; CFU, colony forming unit]

Well identification number	GAMA-PBP identification number	USGS site identification number	California state well number	Date sampled (mm/dd/yyyy)	Number of total coliforms (CFUs)	Number of non-target colonies (CFUs)
K03	S3-MACK-K03	363000119590001	16S/18E-27B1M	01/28/2014	1	0
K05	S3-MACK-K05	363500120090001	15S/17E-31G1M	01/30/2014	5	0
K06	S3-MACK-K06	363500120110001	15S/16E-26H2M	01/29/2014	2	0
K07	S3-MACK-K07	363800120130001	15S/16E-03N1M	04/08/2014	0	1
K07	S3-MACK-K07	363800120130001	15S/16E-03N1M	Duplicate	0	0
K08	S3-MACK-K08	364400120070001	14S/17E-05J1M	01/28/2014	0	0
K09	S3-MACK-K09	364000120080001	14S/17E-29M1M	02/23/2014	1	136
K13	S3-MACK-K13	362500119540001	017S019E22N002M	01/27/2014	0	0
K14	S3-MACK-K14	362400119490001	017S020E32E001M	01/27/2014	0	0
K15	S3-MACK-K15	362600119440001	017S021E18N001M	02/03/2014	19	1
K16	S3-MACK-K16	363000119430001	016S021E30H001M	02/03/2014	3	0
K17	S3-MACK-K17	363100119490001	016S020E20Q002M	02/04/2014	0	0
K19	S3-MACK-K19	364200119570001	014S018E24B001M	02/05/2014	0	0
K20	S3-MACK-K20	364300120000001	014S018E09Q001M	03/06/2014	0	0
K21	S3-MACK-K21	364500120050001	013S017E34H001M	02/05/2014	0	0
K21	S3-MACK-K21	364500120050001	013S017E34H001M	Duplicate	0	0
K22	S3-MACK-K22	364600119540001	013S019E21Q001M	01/29/2014	2	0
K25	S3-MACK-K25	363400119440001	015S020E36J001M	02/26/2014	8	4
K26	S3-MACK-K26	363325119440901	016S021E07E001M	02/04/2014	0	0
K27	S3-MACK-K27	363100119370001	016S021E24H001M	03/04/2014	0	0
K28	S3-MACK-K28	363000119300001	016S023E30G001M	03/18/2014	1	0
K29	S3-MACK-K29	362800119230001	017S024E05K001M	03/17/2014	0	0
K29	S3-MACK-K29	362800119230001	017S024E05K001M	Duplicate	0	0
K30	S3-MACK-K30	363100119290001	016S023E20R001M	02/06/2014	9	8
K31	S3-MACK-K31	363300119310001	016S022E01P001M	02/27/2014	3	0
K32	S3-MACK-K32	363712119394801	015S021E14N002M	04/07/2014	0	0
K32	S3-MACK-K32	363712119394801	015S021E14N002M	Duplicate	0	2
K39	S3-MACK-K39	363700119280001	015S023E16E001M	03/03/2014	0	0
K39	S3-MACK-K39	363700119280001	015S023E16E001M	Duplicate	0	0
K40	S3-MACK-K40	363400119290001	015S023E32J001M	02/25/2014	0	0
K41	S3-MACK-K41	363100119210001	016S024E22C001M	03/04/2014	1	0
K42	S3-MACK-K42	362600119190001	017S024E13L001M	03/17/2014	0	8
K43	S3-MACK-K43	363200119140001	016S025E15A001M	03/05/2014	0	5
K44	S3-MACK-K44	363900119180001	015S024E01J001M	03/05/2014	0	1
K45	S3-MACK-K45	363900119200001	015S024E02F001M	02/25/2014	46	0
K46	S3-MACK-K46	364200119290001	014S023E20F003M	02/24/2014	4	0
K46	S3-MACK-K46	364200119290001	014S023E20F003M	Duplicate	0	3

Table 1.   List of domestic sites sampled for fecal indicator bacteria and number of colony-forming units (CFUs) per 250 milliliters of sample from cultures on MI and mEI media, southeastern San Joaquin Valley, January to April 2014. —Continued

[Well identification number is a shortened form of the GAMA-PBP identification number given in Shelton and Fram (2017). *Escherichia coli* and enterococci were not detected in any of the samples. **Abbreviations**: GAMA-PBP; Groundwater Ambient Monitoring and Assessment Priority Basin Project; USGS, U.S. Geological Survey; mm/dd/yyyy, month/day/year; CFU, colony forming unit]

Well identification number	GAMA-PBP identification number	USGS site identification number	California state well number	Date sampled (mm/dd/yyyy)	Number of total coliforms (CFUs)	Number of non-target colonies (CFUs)
K47	S3-MACK-K47	364600119240001	013S024E30L002M	03/03/2014	98	46
K48	S3-MACK-K48	365000119320001	012S022E35N001M	02/24/2014	0	1
K49	S3-MACK-K49	365200119330001	012S022E22P001M	02/26/2014	6	2
M01	S3-MACK-M01	364600120110001	013S016E25D003M	03/19/2014	38	68
M02	S3-MACK-M02	365400120160001	012S015E12A001M	04/08/2014	0	2
M03	S3-MACK-M03	365600120180001	011S015E27H001M	04/10/2014	9	29
M04	S3-MACK-M04	365700120220001	011S015E19Q001M	04/10/2014	1	3
M12	S3-MACK-M12	364900120030001	013S017E01J001M	03/18/2014	0	0
M17	S3-MACK-M17	370700120210001	009S015E29K001M	03/20/2014	0	0
M25	S3-MACK-M25	365800119530001	011S019E15L001M	03/19/2014	0	0
M28	S3-MACK-M28	370700120040001	009S017E26A001M	04/09/2014	1	1

Table 2.   Genera of bacteria that were identified as target and non-target colonies on MI media where DNA was successfully sequenced from 14 groundwater samples collected from 13 domestic wells in the southeastern San Joaquin Valley from January to April 2014.

[DNA sequences identified more than one potential genera for five of the colonies. Query coverage is the percentage of the sequence length of the colony DNA that is aligned with DNA segments of the BLAST database sequence. Identity is the extent to which the two sequences have the same nucleotide at the same position in an alignment. R, laboratory replicate]

Well identification number	Sequence file name[1]	Genera name	Query coverage (percent)	Identity (percent)
		Genera identified from target colonies		
K28	Batch 2-03	*Pantoea*	100	99
K30	Batch 1-05_R	*Pseudomonas or*	95	99
		Thalassospira	100	99
K30	Batch 1-07	*Pseudomonas or*	95	99
		Thalassospira	100	99
K30	Batch 1-06	*Erwinia*	100	99
K31	Batch 1-09	*Agrobacterium*	100	99
K45	Batch 1-11	*Chryseobacterium*	98	87
K45	Batch 1 -12	*Pseudomonas*	100	99
K46	Batch 1-23	*Chryseobacterium*	99	95
K46	Batch 1-20	*Achromobacter*	100	97
K46	Batch 1-25	*Achromobacter*	100	97
K46	Batch 1 -28	*Cupriavidus*	100	97

Table 2.   Genera of bacteria that were identified as target and non-target colonies on MI media where DNA was successfully sequenced from 14 groundwater samples collected from 13 domestic wells in the southeastern San Joaquin Valley from January to April 2014. —Continued

[DNA sequences identified more than one potential genera for five of the colonies. Query coverage is the percentage of the sequence length of the colony DNA that is aligned with DNA segments of the BLAST database sequence. Identity is the extent to which the two sequences have the same nucleotide at the same position in an alignment. R, laboratory replicate]

Well identification number	Sequence file name1	Genera name	Query coverage (percent)	Identity (percent)
Genera identified from target colonies —Continued				
K49	Batch 1-37	_Tyzzerella or_	99	96
		Streptomyces	99	96
M01	Batch 2-05	_Pseudomonas_	100	99
M01	Batch 2-06	_Pseudomonas_	100	98
M03	Batch 3-08_R	_Acinetobacter_	100	99
M03	Batch 3-09	_Acinetobacter_	100	88
M03	Batch 3-10	_Ochrobactrum_	100	98
M03	Batch 3-11	_Sphingobacterium_	79	99
M04	Batch 3-13	_Cupriavidus_	100	98
Genera identified from non-target colonies				
K09	Batch 1-01	_Chryseobacterium_	99	92
K09	Batch 1-02	_Chryseobacterium_	100	98
K42	Batch 2-01	_Achromobacter_	100	99
K42	Batch 2-02	_Achromobacter_	100	99
K44	Batch 1-10	_Chryseobacterium_	100	100
K46	Batch 1-14	_Curtobacterium_	100	99
K46	Batch 1-16	_Rhodococcus_	100	97
K46	Batch 1-29	_Pseudomonas_	100	99
K48	Batch 1-33	_Sphingobium_	100	95
M01	Batch 2-07	_Pseudomonas or_	96	98
		Thalassospira	100	99
M02	Batch 3-03	_Cupriavidus_	100	99
M02	Batch 3-04	_Stenotrophomonas_	100	98
M03	Batch 3-07	_Brevibacterium_	83	99
M04	Batch 3-12	_Pseudomonas or_	95	99
		Thalassospira	100	99
M04	Batch 3-14	_Mycolicibacterium_	100	100

[1]Sequence files can be found in Soldavini and others, 2021.

Table 3.   Pathogenicity, microbial source, and selected references for genera identified in samples collected from wells in the southeastern San Joaquin Valley from January to April 2014.

[Pathogenicity may vary from species to species within a specified genus. **Abbreviation**: PAH, polycyclic aromatic hydrocarbons]

Well identification number	Genera name	Pathogenicity	Habitat, source	References
Genera identified from target colonies				
M03	*Acinetobacter*	Human pathogen, non-pathogenic	Water, soil, sewage, human skin	Towner, 1997; Guardabassi and others, 1999; Visca and others, 2011
K31	*Agrobacterium*	Plant pathogen	Soil	Goodner and others, 2001; Nester, 2015
K30	*Erwinia*	Plant pathogen	Fruits and vegetables	Hauben and others, 1998; Zhang and Nan, 2014; Gálvez and others, 2015
M03	*Ochrobactrum*	Opportunistic human and animal pathogen	Soil, plants, water, animals, humans	Holmes and others, 1988; Chain and others, 2011; Alonso and others, 2017
K28	*Pantoea*	Plant and opportunistic human pathogen	Plants, seeds, soil, humans, and animals	Gavini and others, 1989; Delétoile and others, 2009; Dutkiewicz and others, 2016
M03	*Sphingobacterium*	Non-pathogenic, uncommon human pathogen	Blood, soil, plants, food	Yabuuchi and others, 1983; Grimaldi and others, 2012; Wauters and others, 2012
K49	*Streptomyces*	Antimicrobial, plant pathogen	Soil, marine sediments, agricultural places, decaying vegetation	Nishikawa and Kobayahi, 2009; Labeda, 2010; Arasu and others, 2014
Genera identified in target and non-target colonies				
K42, K46	*Achromobacter*	Opportunistic human pathogen, non-pathogenic	Soil, water	Duggan and others, 1996; Garrity and others, 2005; Swenson and Sadikot, 2015
K09, K44, K46	*Chryseobacterium*	Non-pathogenic, opportunistic human pathogen	Water, soil, plants, sludge, dairy products	Montero-Calasanz and others, 2013; Lin and others, 2019
M02, M04, K46	*Cupriavidus*	Potential human pathogen, Non-pathogenic	Soil	Makkar and Casida, 1987; Vandamme and Coenye, 2004 Kirsten and others, 2011; Langevin and others, 2011
M01, M04, K30	*Pseudomonas*	Opportunistic human pathogen, plant pathogen, non-pathogenic	Soil, garbage, water, plants, humans	Mo and Gross, 1991; Iglewski, 1996; Elomari and others, 1997; Hirano and Upper, 2000; Beiki and others, 2016; Kwak and others, 2016
Genera identified from non-target colonies				
M03	*Brevibacterium*	Non-pathogenic, opportunistic human pathogen,	Soil, dairy products, humans, or animals	Gruner and others, 1993, 1994; Gavrish and others, 2004; Bal and others, 2015
K46	*Curtobacterium*	Plant pathogen, non-pathogenic, opportunistic human pathogen	Soil, plants, leaf litter	Funke and others, 2005; Aizawa and others, 2007; European and Mediterranean Plant Protection Organization, 2011; Chase and others, 2016
M04	*Mycolicibacterium*	Non-pathogenic, opportunistic human pathogen	Water, soil	Kusunoki and Ezaki, 1992; Wallace and others, 2005; Gupta and others, 2018
K46	*Rhodococcus*	Non-pathogenic, opportunistic human pathogen	Soil, water	McLeod and others, 2006; Baba and others, 2009; Park and others, 2011; Bagdure and others, 2012
K48	*Sphingobium*	Non-pathogenic; degrades PAHs, pesticides	Soil, water	Takeuchi and others, 2001; Gai and others, 2011; Chen and others 2013; Verma and others, 2014
M02	*Stenotrophomonas*	Opportunistic human pathogen, non-pathogenic	Soil, plants, water, animals	Ryan and others, 2009; Brooke, 2012

Table 3. Pathogenicity, microbial source, and selected references for genera identified in samples collected from wells in the southeastern San Joaquin Valley from January to April 2014. —Continued

[Pathogenicity may vary from species to species within a specified genus. **Abbreviation**: PAH, polycyclic aromatic hydrocarbons]

Well identification number	Genera name	Pathogenicity	Habitat, source	References
Other genera identified from gene sequences but not likely to be present				
M01, K30	*Thalassospira*	Possible therapeutic applications	Marine water, plants	López-López and others, 2002; Liu and others, 2007; Tsubouchi and others, 2014
K49	*Tyzzerella*	Potential pathogen	Fecal	Yutin and Galperin, 2013; Ascher and Reinhardt, 2018; Fomenky and others, 2018

Discussion

Bacteria Identification

Culture media are designed to promote growth of desired bacteria while minimizing growth of non-target bacteria. The MI media contains an antibiotic to supress the growth of non-target bacteria; however, the antibiotic does not prevent the growth of all non-target bacteria. For example, non-target colonies can grow when there is an unusually high population of bacteria in the sample (Brenner and others, 1993; Alonso and others, 1996), and the results from this study are consistent with that behavior. The median number of total colonies in samples having eight or more non-target colonies is significantly greater than the median number of total colonies in samples having five or fewer non-target colonies (Wilcoxon rank-sum test, $p < 0.001$; data in table 2). Growth of non-target colonies also could indicate that the antibiotic in the MI media was becoming ineffective. However, Control cultures grown on each lot of MI media used indicated the antibiotic was functioning properly. Whether the bacterium is a target colony or non-target colony is less important than whether the bacterium is an indicator that pathogenic bacteria may be present in the groundwater.

The MI test is known to be less than 100 percent accurate. Maheux and others (2008) found a false-negative rate of 26 percent and a false-positive rate of 3.4 percent for TCs. Brenner and others (1993) found a false-negative rate of 4.3 percent and a false-positive rate of 6.9 percent. Four genera of bacteria, *Achromobacter*, *Chryseobacterium*, *Cupriavidus*, and *Pseudomonas*, were identified both in target and non-target colonies from samples. Some *Pseudomonas* and *Cupriavidus* species may have positive ß-D-galactosidase reactions (Mo and Gross, 1991; Kirsten and others, 2011), which could explain why these bacteria may form colonies that fluoresce blue. Some of the colonies for these four genera could have contained more than one species. An additional species could have caused blue fluorescence that was not identified by the PCR analysis.

Despite that fact that the FlashGel results indicated that amplification had been successful for samples from 59 colonies, samples from only 34 colonies were successfully sequenced by GeneWiz. The primers had not properly annealed in the samples from the other 25 colonies. Use of primers other than or in addition to the 8F and 787R primers might have resulted in better success. Lengthening the time or a using different temperature for the annealing process in the thermocycler may also have improved the results of this process. Errors in oligonucleotide sequencing also are possible. Accumulation of polymorphisms in conservative regions of the 16S gene can lead to failure in primer annealing (Hall, 1994). Widely used primers may not be suitable for a small group of bacteria. For two samples, the quality of the DNA was too poor for sequencing. The lower certainty of genera identification for some of the bacteria primarily was due to poor condition of the DNA or to high background noise, which limited the contiguous read length of base pairs. For most of the genera with identity at less than 99 percent certainty, poor condition of the sequence sample resulted in sequences with fewer bases. Potential bias in the success of colony sequencing could not be evaluated because the success rate was about one-third both for target colonies and non-target colonies.

Uncertainties in the identification of a bacteria can result from similarities in the gene sequence in the area amplified by the universal primers. This study had two samples for which the possible genera were not closely related but had similar gene sequences in the area amplified. *Pseudomonas* and *Thalassospira* were possible candidate genera for two colonies from the K30 sample and one colony from the M01 sample. *Pseudomonas* had lower query coverages than *Thalassospira*, although the certainty of identity percentages was similar (table 2). A search of the literature for the two genera, however, showed that *Thalassospira* is found in saline waters because it is strictly halophilic (López-López and others, 2002; Liu and others, 2007; Tsubouchi and others, 2014), indicating that *Pseudomonas* is the more likely genus of the two potential candidates. *Tyzzerella* and *Streptomyces* were possible candidate genera for a target colony from the K49 sample. *Tyzzerella* are anaerobic bacteria that are present in fecal matter and are often found in human intestinal systems (Holdeman and Moore, 1974; Ascher and Reinhardt, 2018; Fomenky and others, 2018), and *Streptomyces* are aerobic, filamentous, bacteria present in soil (Arasu and others, 2014). The K49 water sample was highly oxygenated (Shelton and Fram, 2017), which indicates that the aerobic bacterium *Streptomyces* is the more likely candidate of the two genera.

Pathogenicity of Identified Bacteria

A review of relevant literature revealed that one genus identified included at least one species that was a human pathogen and 12 more genera included species that could be opportunistic or potential human pathogens (table 3). Most of the potential pathogenic bacteria were not coliforms, however, based on the MI description of a coliform used in this report. Only three coliform genera were target bacteria that could be opportunistic human pathogens. The other coliform genera were either non-pathogenic or were plant pathogens. One coliform genus, *Pantoea*, includes species that are both a plant pathogen and an opportunistic human pathogen. This study confirms that the detection of TCs does not always indicate the presence of potentially pathogenic bacteria, nor does the non-detection of TCs always indicate the absence of potentially pathogenic bacteria (table 3). The results support the hypothesis that the MI test may be useful in determining if groundwater is contaminated with pathogenic bacteria.

Some non-target bacteria genera identified were pathogenic as well. Nine non-target genera included species that could be human pathogens or opportunistic human pathogens (including the four genera that manifested as both target and non-target colonies). This indicates the MI test is useful in determining the possibility of water contaminated with pathogenic bacteria even if the bacteria are not target bacteria.

One non-target genus, *Sphingobium*, was non-pathogenic. Interestingly, *Sphingobium* is known to degrade polycyclic aromatic hydrocarbons (PAHs) as well as pesticides (Gai and others, 2011); therefore, the presence of *Sphingobium* might help reduce concentrations of contaminants in the aquifer. It could, however, also indicate point or non-point source pollution of pesticides or PAHs upgradient from the well. One target genus that is a plant pathogen, *Streptomyces*, also has antimicrobial characteristics that may be beneficial to humans (Nishikawa and Kobayahi, 2009; Arasu and others, 2014).

Some genera consist of non-pathogenic and potentially human pathogenic species. In this study, the species present in the water samples could not be definitively identified to determine pathogenicity. Given the number of genera identified with potentially pathogenic species, the results indicate that pathogenic bacteria could be widely distributed throughout the study area. The FIBs detected in wells can be removed by disinfecting the well or treating water used for consumption.

Potential Sources of Identified Bacteria

Coliform bacteria are used as indicator bacteria because they live in the intestines of warm-blooded animals and may be an indicator of possible fecal contamination; however, coliforms also can live in environments not contaminated by feces or sewage (Brennan and others, 2010). Coliforms are present in water, soil, garbage, skin, nonfecal bodily fluids, and on plants (DeSimone and others, 2009). Aerobic, Gram-negative rod bacteria are widely distributed in soils and shallow groundwater and include such genera as *Pseudomonas*, *Flavobacterium*, *Rhizobium*, *Alcaligenes*, and *Azotobacter* (Chapelle, 2001). Because of the many sources of bacteria, contamination of domestic wells by bacteria is common (DeSimone and others, 2009). Knowing the source or potential sources of bacteria can help well owners determine if bacterial contamination of well water is a continuous or occasional threat. Well owners can remove bacterial contamination from the well by disinfecting the well as needed or by removing the source of contamination.

Bacteria identified in this study were present in a variety of media. Of the 17 genera, 16 were present in soil; 9 were present in water; 9 were present in or on plants, fruits or vegetables; 6 were present on humans or animals; and 4 were present in other media such as dairy products, garbage, and blood (table 3). Many bacteria were present in more than one medium. Not surprisingly, almost all the genera identified are naturally present in soil or groundwater, indicating that the primary source of the bacteria in most wells is the soil and not septic systems. Bacteria from other sources, such as blood, feces, garbage, or dairy products, could enter the groundwater system through septic systems or infiltration from the ground surface with precipitation, irrigation, or other water usage. The presence of soil bacteria in samples from wells could indicate problems with the well seal allowing surface water to flow into the well or relatively short travel times between recharge and the well (that is, no attenuation of bacteria by percolation through the aquifer). Many of the domestic wells in this study are in areas of the San Joaquin Valley dominated by vertical groundwater flow paths and coarse-grained sediments that have relatively fast recharge rates (Faunt, 2009). The wells are relatively shallow with median depth of 55 m below land surface, and the median depth to water is 25 m below land surface; thus, even in the absence of short-circuiting of flow paths through insufficient well seals, the distance the water traveled through the aquifer to the well was likely relatively short. Nearly all of the samples were primarily composed of modern-age water that was recharged into the aquifer between 1 and 70 years prior to sample collection (Faulkner and Jurgens, 2019). The combination of aquifer conditions and well characteristics favoring rapid recharge and the young age of the water tapped by the wells indicates that these wells may be susceptible to surface contamination.

All 13 genera identified in this study that could have potentially human pathogenic species can be found naturally in soils, plants, or groundwater, but animals are also potential sources for 6 of these 13 genera. The non-pathogenic and plant-pathogenic bacteria are found naturally in soils, plants, and groundwater. The presence of TCs and non-target bacteria growth on MI media indicates the possible presence of pathogens, but the pathogens may not be of fecal or even mammalian origin. The TCs and other detected pathogens could be removed by disinfecting wells or identifying and fixing issues with well seals.

Conclusions

Domestic wells are an important source of drinking water in the southeastern San Joaquin Valley, California,

and microbial contamination in those wells could cause water-borne diseases. Forty-two domestic wells were sampled in 2014 in the Chowchilla, Madera, and Kings groundwater subbasins of the San Joaquin Valley for a study to identify the species of bacteria present in groundwater used by domestic wells. The study was part of the Groundwater Ambient Monitoring and Assessment Program Priority Basin Project (GAMA-PBP), a cooperative study between the U.S. Geological Survey and the California State Water Resources Control Board.

The MI test, a membrane filtration method for the chromogenic/fluorogenic detection of total coliforms and *Escherichia coli*, was used to test for the presence of coliform bacteria. Samples from almost two-thirds of the wells analyzed (27 of 42) had microbial growth on MI media. The microbial growth for 20 wells included target coliform colonies and included non-target colonies for 18 wells. Polymerase chain reaction analysis (PCR) and deoxyribonucleic acid (DNA) sequencing were used to identify genera of bacteria in 34 of the target and nontarget colonies from 13 wells. Gene sequences obtained using the Sanger method were entered into the basic local alignment search tool (BLAST) database, and 17 genera of bacteria were identified. Of these 17 genera, 13 genera include species that are human pathogens or opportunistic human pathogens. More than half the genera with possible human pathogenic bacteria were isolated from non-target colonies that grew on the MI medium; therefore, the presence of total coliforms is not a strong indicator of the presence of human pathogens. The MI test was useful in determining the possibility that water may be contaminated with pathogenic bacteria, however, even if the bacteria are not coliforms. A couple of the genera identified included bacteria that could be beneficial to humans. *Streptomyces* has antimicrobial characteristics, and *Sphingobium* can degrade pesticides and polycyclic aromatic hydrocarbons (PAHs).

Microbial contamination in domestic wells does not necessarily mean that the source of the contamination is fecal. Based on a literature search, all 13 of the genera that include possible human pathogens are natural inhabitants of soils, plants, or groundwater. Six of the genera also have animals and humans as one of the potential sources. All the non-pathogenic and plant pathogenic bacteria are found naturally in soils, plants, or groundwater. This indicates that the sources of bacteria to these domestic wells are most likely soil, not human fecal matter from septic systems. Disinfecting the well or fixing leaky well seals can help remove bacterial growth identified from analyzed samples of well water. Future studies could investigate the relation of microbial communities with water quality and other environmental factors.

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