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Coliform detection in cheese is associated with specific cheese characteristics, but no association was found with pathogen detection

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ABSTRACT

Coliform detection in finished products, including cheese, has traditionally been used to indicate whether a given product has been manufactured under unsanitary conditions. As our understanding of the diversity of coliforms has improved, it is necessary to assess whether coliforms are a good indicator organism and whether coliform detection in cheese is associated with the presence of pathogens. The objective of this study was (1) to evaluate cheese available on the market for presence of coliforms and key pathogens, and (2) to characterize the coliforms present to assess their likely sources and public health relevance. A total of 273 cheese samples were tested for presence of coliforms and for *Salmonella*, *Staphylococcus aureus*, Shiga toxin-producing *Escherichia coli*, *Listeria monocytogenes*, and other *Listeria* species. Among all tested cheese samples, 27% (75/273) tested positive for coliforms in concentrations >10 cfu/g. Pasteurization, pH, water activity, milk type, and rind type were factors significantly associated with detection of coliforms in cheese; for example, a higher coliform prevalence was detected in raw milk cheeses (42% with >10 cfu/g) compared with pasteurized milk cheese (21%). For cheese samples contaminated with coliforms, only water activity was significantly associated with coliform concentration. Coliforms isolated from cheese samples were classified into 13 different genera, including the environmental coliform genera *Hafnia*, *Raoultella*, and *Serratia*, which represent the 3 genera most frequently isolated across all cheeses. *Escherichia*, *Hafnia*, and *Enterobacter* were significantly more common among raw milk cheeses. Based on sequencing of the housekeeping gene *clpX*, most *Escherichia* isolates were confirmed as members of fecal commensal clades of *E. coli*. All cheese samples tested negative for *Salmonella*, *Staph. aureus*, and Shiga toxin-producing *E. coli*. *Listeria* spp. were found in 12 cheese samples, including 5 samples positive for *L. monocytogenes*. Although no association was found between coliform and *Listeria* spp. detection, *Listeria* spp. were significantly more likely to be detected in cheese with the washed type of rind. Our data provide information on specific risk factors for pathogen detection in cheese, which will facilitate development of risk-based strategies to control microbial food safety hazards in cheese, and suggest that generic coliform testing cannot be used to assess the safety of natural cheese.
Key words: cheese, coliform, pathogen, fecal contamination, pasteurization

INTRODUCTION

The coliform bacteria are a nontaxonomic classification that by definition describes a group of gram-negative, facultative anaerobic rod-shaped bacteria that are able to ferment lactose with production of acid and gas within 48 h at 32 to 35°C (Davidson et al., 2004). This simple concept of coliforms was developed more than 100 yr ago to test water for fecal contamination. The coliform test, because of its convenience, was quickly adopted by dairy and other branches of the food industry. Today, generic coliform testing is still used in the U.S. dairy industry to indicate unsanitary conditions in which product was manufactured, including postprocessing contamination and, much less likely, the possibility of pasteurization failure. Because coliforms, and gram-negative bacteria in general, are inactivated by pasteurization, using a coliform test in fluid milk production provides some value even though coliforms are responsible for only a part of the spoilage caused by gram-negative bacteria (Martin et al., 2011). Although the generic coliform test is still used in cheese production to indicate unsanitary manufacturing conditions, its usefulness in this product is increasingly being questioned (Strongin, 2015).

Two general misconceptions exist with regard to use of coliforms as indicators, which contribute to possible misinterpretations of coliform detection in cheese. The first is that coliforms exclusively represent bacteria

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coming from a fecal environment. Leclerc and colleagues (2001) separated coliforms into 3 groups, including (1) psychrotolerant environmental coliforms, (2) thermotolerant fecal coliforms, and (3) ubiquitous coliforms that also include some thermotolerant coliforms. Genera such as *Serratia*, *Hafnia*, *Rahnella*, *Buttiauxella*, and *Leclercia* represent environmental coliforms and were shown recently to include at least some strains able to grow in milk at refrigeration temperatures (Masiello et al., 2016). Typical representatives of ubiquitous coliform genera are *Enterobacter* and *Citrobacter*. Both genera include several species that can be found in many known environments, including milk and cheese (Leclerc et al., 2001; Coton et al., 2012; Quigley et al., 2013; Masiello et al., 2016). Fecal coliforms represent only a small proportion of the coliform group, and several different reports suggest that *Escherichia coli* is the only coliform to exclusively represent the fecal environment (Edberg et al., 2000; Stevens et al., 2003; Paruch and Maehlum, 2012). Even though *E. coli* is characterized as being associated with fecal sources, specific bacterial lineages that are not associated with fecal sources have also been identified within the genus *Escherichia*; representatives of these non-fecal-associated lineages are often almost indistinguishable from *E. coli* (Walk et al., 2009; Luo et al., 2011; Oh et al., 2012). Walk et al. (2009) analyzed 22 housekeeping genes and identified 5 new phylogenetic clades of *Escherichia*, of which clades III, IV, and V were isolated from freshwater beaches, suggesting adaptation to the environment outside the intestinal tract of a warm-blooded host.

The second misconception about coliforms is that they can be used as index organisms. By definition, an index organism is an organism whose presence relates to the possible occurrence of ecologically similar pathogens (Mossel et al., 1995; Buchanan, 2000; Kornacki, 2011). Coliforms can be found in the majority of known environments and consequently cannot represent a specific ecological niche of any pathogen. Expectations of coliforms to yield information about the presence of pathogens is again connected to coliforms being perceived as exclusive representatives of the fecal environment, which is further known as a common source of foodborne pathogens.

With regard to water quality, the relevance of coliforms as indicators of fecal contamination and presence of pathogens has already been questioned several times in the past (Stevens et al., 2003; Wu et al., 2011). A similar comprehensive evaluation of coliforms and pathogens in cheese is needed to evaluate the relevance of coliforms as indicator organisms in this food product. Therefore, the objectives of this study were (1) to evaluate cheese available on the market for presence of coliforms and key pathogens, and (2) to characterize the coliforms present to assess their likely sources and public health relevance.

MATERIALS AND METHODS

Collecting and Handling Cheese Samples

Cheese samples were collected from market sources throughout New York State during 2014 and 2015, following a convenient and stratified sampling plan; stratification was performed to ensure representation of raw and pasteurized milk cheeses as well as representation of different cheese categories manufactured from cow, sheep, and goat milks. The 273 cheese samples were selected to capture the diversity of cheese present on the market without the attempt to capture a market basket that represents consumption quantities of different cheese categories. Among these samples, 213 represented natural cheese products that were sampled only once during the study. The remaining samples included instances in which the same cheese product was sampled more than once from a given processor representing either 2 (n = 21) or 3 (n = 6) different production dates. Among the 273 cheese samples, 88 and 185 represented raw milk cheese and pasteurized milk cheese, respectively. Cheese samples were manufactured from cow (n = 125), goat (n = 75), sheep (n = 62), and mixed milks (n = 11). Samples were selected to represent different cheese categories and rind types to ensure inclusion of cheeses representing a wide range and different combinations of pH and water activity. The majority of cheese samples were purchased in grocery shops and supermarkets located in New York State as well as directly from cheese producers, specialty shops, and wholesale distributors. Cheeses sampled were manufactured in the United States (n = 137), as well as 13 other countries (n = 136), including Canada, New Zealand, Israel, and European Union countries (Supplementary Table S1; http://dx.doi.org/10.3168/jds.2016-11112). Approximately 50% of the US cheese samples (n = 68) were manufactured in New York State.

The cheese samples were either prepackaged or packaged in food-grade packing material before being transported in coolers to Cornell University (Ithaca, NY). Cheese samples were held at 4°C until the start of testing. This period was never longer than 7 d for whole cheese wheels and prepackaged cheese samples and never longer than 24 h for cut and packaged cheese samples. Each cheese sample was homogenized by hand in a sterile Whirl-Pak bag (Nasco, Fort Atkinson, WI), and four 25-g aliquots of each cheese sample were weighed into separate sterile Whirl-Pak bags. Cheese aliquots were individually used to determine the concentration of coliforms, as well as presence of *Salmonella*, *Staphy-*