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PSEUDOMONAS AERUGINOSA AND *PSEUDOMONAS* SPP. ISOLATED FROM FRESH *MINAS* CHEESES IN RIO DE JANEIRO

Newton Takeshi Okuno [1*], Isabela da Rocha Freire [1], Cristiane Rodrigues Silva [1], Victor Augustus Marin [1]

1. Federal University of the State of Rio de Janeiro (UNIRIO), Nutrition School, Dr. Xavier Sigaud Street, 290, Urca - Rio de Janeiro/RJ - Brazil, 22240-040.
*newton.takeshi@gmail.com
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ABSTRACT

Pseudomonas aeruginosa is an opportunistic pathogen among the most prevalent nosocomial microorganisms and is particularly relevant to the health of infants, children and immunocompromised persons. *P. aeruginosa* is linked to gastroenteritis and causes the Shanghai fever with necrotizing enteritis and sepsis in infants and children and, although rare, necrotizing enteritis in adults. Cheeses with high moisture content are issues of concern to the health of that population as the already present pathogens can easily grow. In this work, *P. aeruginosa* and *Pseudomonas* spp. strains were found, respectively, in 04 (12.1%) and 19 (57.6%) of 33 samples of fresh *Minas* cheese, a traditional Brazilian cheese, acquired from May 2015 to January 2016 in Rio de Janeiro city. Antimicrobial susceptibility testing of 03 *P. aeruginosa* strains was performed with ticarcillin, ticarcillin-clavulanic acid, ceftazidime, cefepime, aztreonam, imipenem, meropenem, gentamicin, ciprofloxacin and colistin. Although no resistance to these antimicrobials was found, good manufacturing and hygienic practices in dairy plants are paramount to ensure food safety and quality. Furthermore, surveillance of antibiotic resistance and opportunistic pathogens with resistance genes in the dairy chain is useful to control the spread of antibiotic resistance.

KEYWORDS: Antimicrobial susceptibility; Non-fermentative Gram-negative bacilli; PCR

INTRODUCTION

The Brazilian fresh *Minas* cheese is a traditional cheese widely consumed in Brazil. It is a white, soft, non-ripened and salted cheese with high moisture content made with pasteurized milk. The use of pasteurized milk on fresh *Minas* cheese-making process is mandatory and enforced by the Ordinance n. 352/1997 of the Ministry of Agriculture and Supply of Brazil (BRASIL, 1997). The current Brazilian legislation for this type of cheese establishes microbiological limits for *Salmonella*, *Listeria monocytogenes*, coagulase-positive staphylococci, coliform bacteria at 30ºC and 45ºC, yeasts and molds (BRASIL, 1996, 2001).

From 2000 to 2010, 239 gastroenteritis outbreaks linked to dairy products were reported in the state of Sao Paulo, Brazil, but only 79 (33.0%) had the etiological agent identified. "*Staphylococcus aureus* (23.9%), *Escherichia coli* (16.4%), *Salmonella* (14.9%), *Shigella* (11.9%), *Bacillus cereus* (9.0%), *Brucella* (1.5%), *Clostridium botulinum* (1.5%), *Pseudomonas* (1.5%) and other *Enterobacteriaceae* (19.4%)" were responsible for 67 of those outbreaks (MERUSSI et al., 2012).

Pseudomonas spp. of environmental origin is a major problem in Brazilian dairy farms due to poor hygiene and raw milk storage conditions (CAPODIFOGLIO et al., 2016). Water, milkers' hands, teat surface, teat cups, expansion tank, sieves and sponges are common sources of *Pseudomonas* (CAPODIFOGLIO et al., 2016; NUCERA et al., 2016). Though *Pseudomonas* is neither listed on the fresh cheese microbiological criteria nor the most critical microorganism isolated in foodborne outbreaks, *Pseudomonas aeruginosa* is an important opportunistic pathogen for infants, children and immunocompromised persons (CHENG et al., 2009; CHUANG et al., 2017; DE VICTORICA and GALVÁN, 2001; HUNTER and ENSIGN, 1947; ZHANG et al., 2012).

P. aeruginosa is usually linked to clinical and subclinical mastitis in cows (BHATT et al., 2012; FERNANDES et al., 2009), but it is also one of the most prevalent nosocomial microorganisms, mainly associated with soft tissue, respiratory and urinary tract infections, and bacteremia, and often displays a multidrug resistance phenotype (GELLATLY and HANCOCK, 2013). Carbapenem-resistant *P. aeruginosa* is currently ranked as a pathogen with critical priority for research and development of new antibiotics (WHO, 2017).

Regarding the gastrointestinal tract, *P. aeruginosa* is linked to gastroenteritis in both immunocompromised and healthy infants and children, causing the Shanghai fever with necrotizing enteritis and sepsis, and, although rare, necrotizing enteritis in adults (CHENG et al., 2009; CHUANG et al., 2017; DE VICTORICA and GALVÁN, 2001; GANGEMI et al., 2017; HUNTER and ENSIGN, 1947; ZHANG et al., 2012). The first report of the Shanghai fever in South America was made in November 2018 (PENTEADO et al., 2018).

Intensive care unit patients with *Pseudomonas* colonization of the upper gastrointestinal tract have a mortality rate as high as 70%, higher than the mortality rate of 26% in non-colonized patients (MARSHALL et al., 1993). Moreover, prior *P. aeruginosa* rectal colonization seems to precede infection in intensive care unit patients (GÓMEZ-ZORRILLA et al., 2015). Patients with severe systemic inflammatory response syndrome have altered gut flora with higher *Pseudomonas* counts than healthy people (SHIMIZU et al., 2006), and patients with irritable bowel syndrome have a higher prevalence of *P. aeruginosa* (KERCKHOFFS et al., 2011; SHUKLA et al., 2015).

In vivo studies have shown that a multidrug-resistant strain heightened inflammatory responses in mice harboring a human gut microbiota with subacute ileitis (HEIMESAAT et al., 2019) and that some strains can translocate from the gastrointestinal tract to the blood of mice with induced neutropenia (KOH et al., 2005). Some *P. aeruginosa* clinical isolates can also disrupt human intestinal epithelial cells, which may lead to gut-derived sepsis (ZABORINA et al., 2006).

P. aeruginosa outbreaks in hospitals are usually linked to cross-contamination between patients and common environmental reservoirs - tap water, sinks and drinking water fountains (COSTA et al., 2015; ROGUES et al., 2007; ZHOU et al., 2016). However, when there are no positive samples in the ward environment, food and drugs could be the possible sources of *P. aeruginosa* (SHOOTER et al., 1969).

Genomic studies show that clinical and environmental strains have highly conserved genomes, despite their diversity on pathogenicity, virulence and motility (GROSSO-BECERRA et al., 2014; VALOT et al., 2015; WOLFGANG et al., 2003). This diversity may be explained by the expression of quorum sensing-regulated proteins and virulence factors, which are heavily influenced by the environment, nutritional

conditions and host factors (GROSSO-BECERRA et al., 2014; WELSH and BLACKWELL, 2016).

In this work, we assessed the presence of the genus *Pseudomonas* and of *P. aeruginosa* in fresh *Minas* cheeses available in retail stores in Rio de Janeiro city by using polymerase chain reaction and the antimicrobial susceptibility of *P. aeruginosa* strains isolated from these cheeses.

MATERIAL AND METHODS

Sampling

From May 2015 to October 2015, 17 samples of different brands of individually packed fresh *Minas* cheese were acquired from retail stores in Rio de Janeiro city, Brazil, and, from October 2015 to January 2016, 16 samples of the same brands from the first sampling were acquired from retail stores. One manufacturer discontinued one of its brands after the first sampling. All samples had the Brazilian Federal Inspection Seal on their labels, meaning that all manufacturers complied with the use of pasteurized milk on their production.

Sampling followed the requirements of the Brazilian food microbiological standards, Brazilian National Health Surveillance Agency – Board of Directors Resolution No. 12/2001 (BRASIL, 2001). Sample preparation followed the sampling procedures for paste food products of the Brazilian Official Analytical Methods for Microbiological Analysis for Control of products of Animal origin and Water, Normative Instruction MAPA No. 62/2003 (BRASIL, 2003), with slight modifications.

Culturing

A portion of 25.0 ± 0.2 g of homogenized cheese was incubated in 225 ml Gram-negative (GN) broth (HiMedia, India) for 24 hours at 35ºC. An aliquot of 1.0 ml of the enriched culture was collected to extract the total microbial DNA of the cheese as detailed in section 2.4.

Five cetrimide agar plates (HiMedia, India), a selective and differential medium for *P. aeruginosa*, were streaked with 10 µL of the enriched broth and incubated, based on the technical data, at 35ºC for 24 hours.

Screening for *P. aeruginosa* colonies

Two single presumptive *P. aeruginosa* colonies from each cetrimide agar plate were selected by the morphology and biochemistry of *P. aeruginosa* typical colonies on cetrimide agar: irregular borders, production of fluorescein or hydrosoluble pigments as pyocyanin, pyoverdin, pyorubin and pyomelanin. To avoid losing atypical morphological strains, exceeding colonies were collected when more than two morphological distinct colonies showed fluorescence under UV light at 302 nm or when production of pigments occurred.

Extraction and purification of microbial DNA

Extraction and purification of microbial DNA were performed by using GeneJET Genomic DNA Purification Kit (ThermoScientific, Lithuania) according to the manufacturer's GN bacteria genomic DNA purification protocol.

Identification of bacterial cultures

Amplification of target DNA sequences was performed in a LifeTouch Thermal Cycler (BIOER, China) with primers, listed in Table 1, synthesized by Integrated DNA

Technologies (USA); dNTP set (100 mM) from Ludwig Biotec (Brazil); and Taq DNA polymerase kit from Invitrogen (USA).

Table 1. PCR primers for identification of *Pseudomonas* spp. and *P. aeruginosa*

Target	Gene	Primer	Sequence (5'-3')	Amplicon (bp)	Annealing Temp. (°C)	Reference
P. aeruginosa	O-antigen acetylase gene	PA431CF	CTGGGTCGAAAGGTGGTTGTTATC	232	63	(CHOI et al., 2013)
		PA431CR	GCGGCTGGTGCGGCTGAGTC			
Pseudomonas spp.	16S rRNA gene (S)	PA-GS-F	GACGGGTGAGTAATGCCTA	618	54	(SPILKER et al., 2004)
		PA-GS-R	CACTGGTGTTCCTTCCTATA			

Identification of the genus *Pseudomonas* was carried out in a 25 µl reaction volume containing 2.5 µl PCR Buffer 10X, 0.25 mM of each dNTP, 2.0 mM $MgCl_2$, 0.4 µM of each primer, 1 U Taq polymerase and 2.0 µl of purified DNA (SPILKER et al., 2004). Identification of *P. aeruginosa* was carried out in a 25 µl reaction volume containing 2.5 µl PCR Buffer 10X, 0.2 mM of each dNTP, 1.5 mM $MgCl_2$, 0.8 µM of each primer, 2.0 U Taq polymerase and 2.0 µl of purified DNA (CHOI et al., 2013).

P. aeruginosa INCQS 00099 (ATCC 27853) strain from the Collection of Reference Microorganisms on Health Surveillance – CRMVS, FIOCRUZ-INCQS, Rio de Janeiro/RJ, and sterile water were used as positive and negative PCR controls. PCR products were stained with Blue Green Loading Dye I (LGC Biotecnologia, Brazil) followed by electrophoresis through a 0.8% (w/v) agarose gel Hexapur Bio Lab, Holland) in Tris-Borate-EDTA buffer (Promega, USA) alongside a Mid-Range DNA Ladder (Jena Bioscience, Germany).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of *P. aeruginosa* isolates was performed according to the performance standards for antimicrobial susceptibility testing of the CLSI by using disk diffusion method with an 0.5 McFarland inoculum prepared by direct colony suspension (CLSI, 2015).

The panel consisted of at least one antimicrobial agent from each antimicrobial class from the table of zone diameter and minimal inhibitory concentration interpretative standards for *P. aeruginosa* (CLSI, 2015), prioritizing newer generations of penicillins and cephems: penicillins (4^{th} generation – ticarcillin - 75 µg), β-Lactam/β-Lactamase inhibitor combinations (ticarcillin-clavulanic acid - 75/10 µg), cephems (3^{rd} generation – ceftazidime - 30 µg, 4^{th} generation – cefepime - 30 µg), monobactams (aztreonam - 30 µg), carbapenems (imipenem - 10 µg, meropenem - 10 µg), lipopeptides (colistin - 10 µg), aminoglycosides (gentamicin - 10 µg) and fluoroquinolones (ciprofloxacin - 5 µg). All antimicrobial disks were acquired from Oxoid, England. Results were interpreted according to the table mentioned above (CLSI, 2015).

RESULTS

Detection results for *Pseudomonas* and *P. aeruginosa*

We identified *P. aeruginosa* in samples H1, E2, I2 and J2 (n=4; 12.1%). Electrophoresis gel of PCR products (Figure 1) displays four clear bands of the same size as the positive control *P. aeruginosa* INCQS 00099 (ATCC 27853) strain. Detailed information on these four samples is available in Table 2.

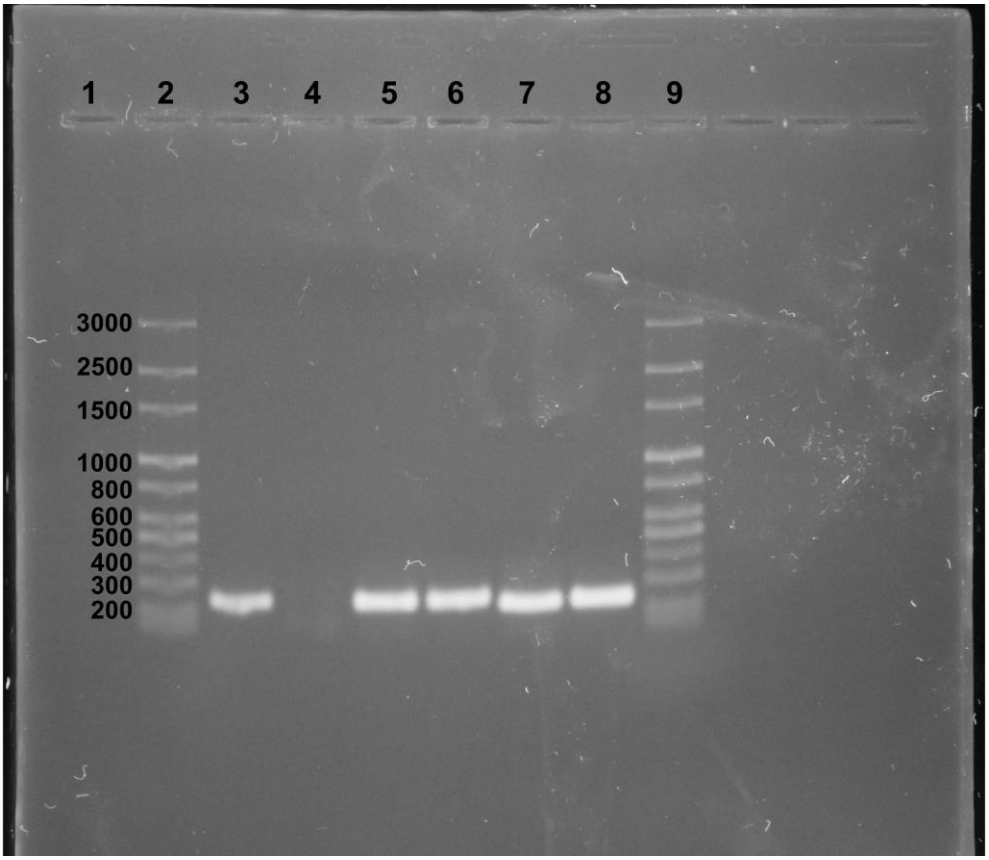

Figure 1. Electrophoresis gel of PCR products - *P. aeruginosa* 0.8 % (w/v) agarose gel, 85 V, 2 hr. Lane 2 and 9 – Mid Range DNA Ladder – Jena Bioscience; Lane 3 – Positive control *P. aeruginosa* INCQS 00099 (ATCC 27853) strain – expected size of 232 bp; Lane 4 – Negative control; Lane 5 – H1 isolate; Lane 6 – E2 isolate; Lane 7 – I2 isolate; Lane 8 – J2 isolate

Table 2. Supplementary data of the samples positive for *P. aeruginosa*

	H1	**E2**	**I2**	**J2**
Date of manufacture	06/29/2015	11/25/2015	10/26/2015	10/20/2015
Season	Winter	Spring	Spring	Spring
Expiration date	07/29/2015	12/14/2015	11/26/2015	11/19/2015
Date of analysis	07/07/2015	12/01/2015	11/03/2015	11/16/2015
Time between manufacture and analysis	9 days	7 days	9 days	28 days
Sampling location (Neighborhood)	Catete	Humaitá	Copacabana	Flamengo
Storage temperature	4.2ºC	7.6ºC	7.2ºC	7.5ºC

The detection results of *Pseudomonas* and *P. aeruginosa* for each of the 33 samples are summarized in Table 3. The total microbial DNA from samples A1 and B1 were lost on sampling. We could not achieve single colonies in sample G2. Therefore, and only for that sample, the full content of each of the five plates was collected.

Table 3. PCR detection of *Pseudomonas* Genus and *P. aeruginosa* in total and isolated DNA [a]

Sample	*Pseudomonas* spp. – Total microbial DNA	*P. aeruginosa* - Total microbial DNA	*Pseudomonas* spp. – Single colonies DNA	*P. aeruginosa* – Single colonies DNA	Ratio Species/Genus
A1	N/A	N/A	0/10 (0%)	0/10 (0%)	-
B1	N/A	N/A	0/10 (0%)	0/10 (0%)	-
C1	-	-	0/10 (0%)	0/10 (0%)	-
D1	-	-	0/10 (0%)	0/10 (0%)	-
E1	+	-	0/10 (0%)	0/10 (0%)	-
F1	+	-	0/10 (0%)	0/10 (0%)	-
G1	-	-	0/10 (0%)	0/10 (0%)	-
H1[b]	-	-	5/8 (63%)	4/8 (50%)	4/5 (80%)
I1	-	-	0/10 (0%)	0/10 (0%)	-
J1	+	-	0/10 (0%)	0/10 (0%)	-
K1	+	-	0/10 (0%)	0/10 (0%)	-
L1	+	-	0/11 (0%)	0/11 (0%)	-
M1	+	-	0/10 (0%)	0/10 (0%)	-
N1	-	-	0/10 (0%)	0/10 (0%)	-
O1	-	-	0/10 (0%)	0/10 (0%)	-
P1	+	-	5/16 (31%)	0/16 (0%)	0/5 (0%)
Q1	+	-	5/15 (33%)	0/15 (0%)	0/5 (0%)
A2	+	-	0/10 (0%)	0/10 (0%)	-
B2	-	-	0/10 (0%)	0/10 (0%)	-
C2	+	-	6/15 (40%)	0/15 (0%)	0/6 (0%)
D2	+	-	6/14 (43%)	0/14 (0%)	0/6 (0%)
E2	+	-	6/13 (46%)	6/13 (46%)	6/6 (100%)
F2	+	-	0/12 (0%)	0/12 (0%)	-
G2	-	-	3/5 (60%)	0/5 (0%)	0/3 (0%)
H2	-	-	0/10 (0%)	0/10 (0%)	-
I2	+	+	11/11 (100%)	11/11 (100%)	11/11 (100%)
J2	+	-	9/15 (60%)	5/15 (33%)	5/9 (56%)
K2	-	-	0/10 (0%)	0/10 (0%)	-
L2	-	-	0/10 (0%)	0/10 (0%)	-
M2	+	-	1/11 (9%)	0/11 (0%)	0/1 (0%)
O2	-	-	0/13 (0%)	0/13 (0%)	-
P2	-	-	0/10 (0%)	0/10 (0%)	-
Q2	-	-	1/11 (9%)	0/11 (0%)	0/1 (0%)
Total	16/31 (52%)	1/31 (3%)	58/360 (16%)	26/360 (7%)	26/58 (45%)

[a] N/A: Not analyzed; -: Absence; +: Presence

[b] Two single colonies from sample H1 did not grow when re-suspended in GN broth.

Antimicrobial susceptibility testing results

None of the *P. aeruginosa* isolates displayed resistance to the tested antimicrobials, as shown in Table 4.

Table 4. Clinical Breakpoints and results of antimicrobial susceptibility test of *P. aeruginosa* strains [a]

Antimicrobial tested	Breakpoints for the Zone Diameter Interpretative Criteria (mm)	H1	E2	I2
Ticarcillin (75 µg)	S≥ 24 / I: 16-23 / R ≤ 15	S	S	S
Ticarcillin-clavulanate (75/10 µg)	S≥ 24 / I: 16-23 / R ≤ 15	S	S	S
Ceftazidime (30 µg)	S≥ 18 / I: 15-17 / R ≤ 14	S	S	S
Cefepime (30 µg)	S≥ 18 / I: 15-17 / R ≤ 14	S	S	S
Aztreonam (30 µg)	S≥ 22 / I: 16-21 / R ≤ 15	S	S	S
Imipenem (10 µg)	S≥ 19 / I: 16-18 / R ≤ 15	S	S	S
Meropenem (10 µg)	S≥ 19 / I: 16-18 / R ≤ 15	S	S	S
Gentamicin (10 µg)	S≥ 15 / I: 13-14 / R ≤ 12	S	S	S
Ciprofloxacin (5 µg)	S≥ 21 / I: 16-20 / R ≤ 15	S	S	S
Colistin (10 µg)	S≥ 11 / I: - / R ≤ 10	S	S	S

[a] Due to freeze-thaw issues, all bacterial stock cultures from sample J2 lost their culturability

DISCUSSION

Information on *P. aeruginosa* in fresh *Minas* cheese is scarce and have conflicting results. While one study found no strain in 20 samples acquired from a single dairy processing industry in the middle of the state of Sao Paulo (CASTRO, 2012), another study identified *P. aeruginosa* in all 23 manufactured and 23 homemade cheese samples acquired in the city of Alfenas and surroundings in the state of Minas Gerais (CARVALHO et al., 2009). In the former, all samples were from the same manufacturing plant and, in the latter, the methods for identification of *P. aeruginosa* were poorly described.

Studies with non-ripened cheeses in other countries show a low incidence of *P. aeruginosa*. In Scotland, no strains of *P. aeruginosa* were recovered from dairy samples (HAMOUDA et al., 2008). In Turkey, one study did not recover any strains of *P. aeruginosa* from cheese samples (KESKIN and EKMEKÇI, 2007), while another study recovered *P. aeruginosa* strains from 1.4% (n = 2) of 140 homemade white cheese samples (ARSLAN et al., 2011). In Iraq, however, strains of *P. aeruginosa* were recovered from 75% (n = 30) of 40 soft cheese samples (AL-SHAMMARY, 2015).

The detection rate of 12.1% for *P. aeruginosa* in fresh *Minas* cheese found in this study is comparable to the rejection rate of 10.5% for the water group - mineral water, purified water with salts, ice cubes and bottled water - found in an investigation of food and water microbiological conditions and foodborne disease outbreaks in the Federal District, Brazil, with data from the Central Laboratory of the Federal District (NUNES et al., 2013). The high detection rate suggests that the fresh *Minas* cheese, a cheese with high moisture content, could be a source of *P. aeruginosa* as important as water.

Contamination of cheese made from pasteurized milk is mainly linked to the environment, raw material, handlers and manufacturing process (GOULD et al., 2014; JOHNSON et al., 1990). Raw milk has a complex microbiota that comprises several GN bacteria such as *Pseudomonas*, *P. aeruginosa*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Acinetobacter* and *Stenotrophomonas* (QUIGLEY et al., 2013a, 2013b)

Non-ripened cheeses are more prone to the undesirable growth of pathogens than hard cheeses. Thermal processing of milk is a critical point in non-ripened cheese manufacturing that ensures pathogenic bacteria to be reduced to safe levels. Yet, inadequate pasteurization and post-pasteurization contamination of fluid milk may occur. Culture-based approaches show that the binomial time-temperature used for HTST and UHT pasteurization in Brazil seems to reduce *Pseudomonas* population to

undetectable levels (MILANEZE et al., 2018). However, while evaluation of the efficacy of HTST pasteurization by using culture-based approaches shows a 6-log population reduction of the *Pseudomonas* population, culture-independent methods – quantitative PCR and flow cytometry – show that less than a 2-log reduction is achieved (QUIGLEY et al., 2013a). Therefore, if raw milk from cows with mastitis presenting high levels of *P. aeruginosa* cells is used, pasteurization may not reduce its population to safe levels.

Unsanitary conditions and failures on cleaning and disinfection protocols are a route for post-pasteurization contamination. Cheese vat, cheesecloth, knives, curd cutting knives, brine tank and plastic cups used on cheese making may be a source of *Pseudomonas* (İPEK and ZORBA, 2018). *Pseudomonas* are known for their ability to form biofilm and to adhere to polyethylene and stainless-steel surfaces of storage tanks, holding cells and heat plates of the pasteurizer used in dairy plants (CLETO et al., 2012; PINTO et al., 2015). Moreover, *P. aeruginosa* biofilms may resist to benzalkonium chloride, sodium hypochlorite, or iodophor used even at their recommended concentrations for sanitization routines (PAGEDAR and SINGH, 2015).

Since each positive sample for *P. aeruginosa* – H1, E2, I2 and J2 – was produced in a unique manufacturer plant and had a unique batch number, contamination of the production lines of those manufacturers with *P. aeruginosa* seems to be transient. However, it is not possible to trace back contamination within the production chain with the current data.

The low resistance profile found is similar to those in *P. aeruginosa* strains from cattle. Even though the majority of *P. aeruginosa* strains linked to cattle seem to have a low resistance to antimicrobials (COTON et al., 2012; DECIMO et al., 2016; ODUMOSU et al., 2016; SERRANO et al., 2017), monitoring the development of resistance to antibiotics in the dairy chain is advisable.

Cheese has a diverse microbiota that comprises other *Pseudomonas* species than *P. aeruginosa*, and some *Pseudomonas* species produce heat-resistant proteolytic and lipolytic enzymes linked to the spoilage of dairy products and the formation of off-flavors (ARSLAN et al., 2011; CALDERA et al., 2016; MACHADO et al., 2017). We recovered *Pseudomonas* spp. from 19 cheese samples (57.6%) of which 08 (P1, Q1, C2, D2, E2, I2, J2 and M2) showed positive results for both analyses of the total microbial DNA and from direct plating, 08 (E1, F1, J1, K1, L1, M1, A2 and F2) for analysis of the total microbial DNA only and 03 (H1, G2 and Q2) for analysis from direct plating only. The high detection rate of *Pseudomonas* suggests that this microorganism could be used as an indicator for food safety and quality of fresh *Minas* cheese, as it has already been suggested for the high moisture raw milk mozzarella (MEIER et al., 2018).

Cetrimide agar inhibits the growth of some GN rods and some *Pseudomonas* species other than *P. aeruginosa* (BROWN and LOWBURY, 1965). Since direct plating in cetrimide agar showed no positive results for *Pseudomonas* spp. in samples E1, F1, J1, K1, L1, M1, A2 and F2, we hypothesize that cetrimide agar inhibited the growth of *Pseudomonas* spp. in those samples or *Pseudomonas* spp. cells from those samples were not in a culturable state.

Overall, the total microbial DNA analysis provided better identification of *Pseudomonas* spp. and the culture-based approach provided better identification of *P. aeruginosa*. Analyses of the total microbial DNA showed negative results for *P. aeruginosa* in samples H1, E2 and J2, and for *Pseudomonas* spp. in samples H1, G2 and Q2, while culturable strains of these bacteria were retrieved by using plating method with adequate growth conditions. These data suggest that analyses with only the total

microbial DNA of samples could mislead to false-negative results due to the low concentration of the target DNA.

CONCLUSIONS

The detection rate found of *P. aeruginosa* was similar to that found in water. Therefore, non-ripened cheeses with high moisture content may be an issue of concern to the health of infants, children and immunocompromised persons as the already present pathogens can easily grow. Though none of the three *P. aeruginosa* isolates displayed resistance to the tested antimicrobials, it would be imprudent to suggest that *P. aeruginosa* strains in fresh *Minas* cheese pose no threat to that population. Therefore, good manufacturing and hygienic practices in dairy plants are a top priority to ensure food safety and quality. Likewise, the surveillance of antibiotic resistance and opportunistic pathogens with resistance genes such as *P. aeruginosa* in the dairy chain is useful to control the spread of antibiotic resistance.

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