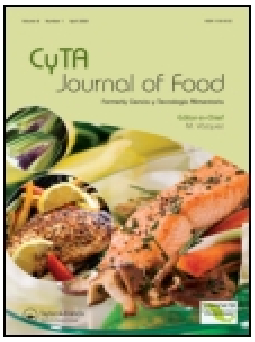

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Microbiological quality and antimicrobial resistance of *Escherichia coli* and *Staphylococcus aureus* isolated from conventional and organic "Arzúa-Ulloa" cheese

Calidad microbiológica y resistencia a antimicrobianos de *Escherichia coli* y *Staphylococcus aureus* aislados a partir de queso "Arzúa-Ulloa" convencional y ecológico

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The presence of *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes* and *Salmonella* spp. was tested in 184 cheese samples included in the Protected Designation of Origin "Arzúa-Ulloa". From these samples, 57 were raw-milk conventional cheese (RCC), 67 were pasteurized-milk conventional cheese (PCC) and the remaining 60 were pasteurized-milk organic cheese (POC). From these samples, a total of 287 *E. coli* and 281 *S. aureus* isolates were analyzed by an agar disk diffusion assay for their resistance to 11 antimicrobial agents. No significant differences were seen in microbiological general acceptance according to European Regulation 2073/2005. Only *L. monocytogenes* showed unsatisfactorily high levels in RCC samples as compared to PCC ($P = 0.0334$) and POC ($P = 0.0138$) samples. Although it was found that both *E. coli* and *S. aureus* isolated from POC samples showed lower resistance to some antimicrobials than isolates from RCC and/or PCC, for other antimicrobials higher resistance rates were found for POC isolates than conventional ones. Thus, the differences in antimicrobial resistance were too ambiguous to recommend a higher use of antimicrobials in conventional dairy herds than in organic ones.

Keywords: organic; antimicrobial; resistance; cheese

La presencia de *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes* y *Salmonella* spp fue investigada en 184 quesos pertenecientes a la denominación de origen "Arzúa-Ulloa". De estas muestras, 57 correspondieron a quesos fabricados a partir de leche cruda convencional (RCC), 67 correspondieron a quesos fabricados a partir de leche pasteurizada convencional (PCC), y las restantes 60 muestras correspondieron a quesos fabricados a partir de leche pasteurizada ecológica (POC). A partir de dichas muestras, se aislaron un total de 287 cepas de *E. coli* y 281 de *S. aureus* y posteriormente se investigó la resistencia a 11 antimicrobianos de estas cepas mediante el método de difusión en agar. No se encontraron diferencias significativas en la aceptabilidad microbiológica de acuerdo a lo establecido en el Reglamento Europeo 2073/2005. Sólo en el caso de *L. monocytogenes*, se observó una mayor de proporción de muestras inaceptables en RCC con respecto a PCC ($P = 0{,}0334$) y POC ($P = 0{,}0138$). Aunque tanto los *E. coli* como los *S. aureus* aislados a partir de POC mostraron menores tasas de resistencia a algunos antimicrobianos que las cepas aisladas a partir de RCC y/o PCC, en el caso de otros antimicrobianos se encontró una mayor tasa de resistencia que en las muestras procedentes de leche convencional. Por lo tanto, las diferencias encontradas en la resistencia a antimicrobianos en función del tipo de leche utilizado en la fabricación del queso resultaron demasiado ambiguas para demostrar un mayor uso de antimicrobianos en la producción de leche convencional que en el caso de la ecológica.

Palabras clave: ecológico; antimicrobiano; resistencia; queso

Introduction

Cheese is currently considered a very safe food. In particular, in the case of cheese made from pasteurized milk, the pasteurization procedure should eliminate the risk of viable vegetative pathogenic organisms (Little et al., 2008). However, pathogenic bacteria transmitted by dairy products, including cheese, are historically responsible for many outbreaks of food origin (De Buyser Dufour, Maire, & Lafarge, 2001). Epidemiologic investigations have demonstrated that pathogenic bacteria responsible for these outbreaks can contaminate cheese by means of raw milk, inadequately pasteurized milk or post-pasteurization contamination with organisms originally derived from raw milk or manufacturing environments (Little et al., 2008). A major concern about bacterial pathogens carried by milk products is that antimicrobials are frequently used for prevention and treatment of bovine infectious diseases such as

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mastitis (Rosati & Aumaitre, 2004; Srinivasan et al., 2007). Furthermore, dry-cow treatment with therapeutic antibiotics is considered an efficient and cost-effective method to prevent subclinical mastitis (Busato, Traschel, Schällibaun, & Blum, 2000). Today, it is generally accepted that antimicrobial resistant bacteria are produced, maintained and disseminated as a result of selection pressure induced by the use of antimicrobial drugs (Aarestrup, 2000; Philips et al., 2004). Thus, as a consequence of the high antimicrobial use in dairy cows, bacterial contaminants carried by milk and milk products often show high levels of antimicrobial resistance (Sandgren, Waller, & Emanuelson, 2008).

In recent years, organic dairy production has drawn increasing attention because of public concerns about food safety, animal welfare and the environmental impacts of intensive livestock systems (Sato, Bartlett, Erskine, & Kaneene, 2005). Nowadays, consumers assign positive characteristics to organic foods, such as the absence of chemical agents, the guarantee of animal welfare during production, environmental friendliness, healthier conditions and better taste as compared to conventional products. On the basis of these beliefs, consumers are willing to pay premium prices for organic foodstuffs (Rosati & Aumaitre, 2004). As a consequence, small dairy farmers whose profit margins are small assume that organic and sustainable production methods are a way to stay profitable (Busato et al., 2000; Sato et al., 2005). In addition, organic farming is also preferred by many producers located in unfavourable locations such as mountainous regions, where organic farming may be the only possibility to survive (Rosati & Aumaitre, 2004).

Despite the consumers' belief that organic foods are healthier than conventional foods, this has not been scientifically proven (Rosati & Aumaitre, 2004). To this end, recent work has suggested that microbiological contamination of organic foods is higher than their conventionally produced counterparts (Bailey & Cosby, 2005; Magkos, Arvaniti, & Zampelas, 2006; Miranda et al., 2007, 2008a; Soonthornchaikul et al., 2006). However, other authors have found that the microbiological quality of organic milk was similar to, or better than, conventional products (Guinot-Thomas, Jonderville, & Laurent, 1991; Lund, 1991; Luukkonen et al., 2005). It was also reported that the antimicrobial resistance of bacteria isolated from organic animal products is usually lower than their conventional counterparts (Cui, Ge, Zheng, & Jianghong, 2005; Miranda et al., 2007, 2008a,b; Soonthornchaikul et al., 2006; Tikofsky, Barlow, Santiesteban, & Schukken, 2003). Thus, a better understanding of the microbiological quality of organic foodstuffs is required to protect consumers and to define accurately the real advantages of consuming organic products. To date, there have been little data about the microbiological quality and antimicrobial resistance of bacteria isolated from organic dairy products such as cheese.

The aims of this work were as follows: (i) to determinate the microbiological quality of conventional raw and pasteurized, as well as organic pasteurized "Arzúa-Ulloa" cheese, a short-ripened cows milk cheese produced in Galicia (NW Spain). (ii) To determinate the antimicrobial resistances of *Escherchia coli* and *S. aureus* isolated from conventional and organic Arzúa-Ulloa cheeses.

Materials and methods

Collection of cheese samples

A total of 184 cheese samples included in the Protected Designation of Origen (PDO) "Arzúa-Ulloa" were taken between 2005 and 2007. Fifty-seven samples were raw-milk conventional cheese (RCC), 67 were pasteurized-milk conventional cheese (PCC) and 60 were pasteurized-milk organic cheese (POC). No "Arzúa-Ulloa" organic cheeses made from raw organic milk were available in the markets at the time of sample collection. All samples were taken from different lots in different days and no more than five samples were taken from each retail market. Not more than 10 samples of the same commercial brand of conventional cheese were taken for this study. For organic cheeses, only five commercial brands certified by Arzúa-Ulloa PDO and an organic official agency were found. Thus, 12 samples were taken from each brand, all of them from different fabrication lots.

Microbiological analyses and isolation procedure

Escherichia coli, *Staphylococcus aureus*, *Salmonella* spp. and *Listeria monocytogenes* were detected and measured on the basis of criteria established in European Regulation 2073/2005 (European Commission, 2005). As no criteria have been established for *E. coli* contamination in raw cheese, the criteria established for *E. coli* in cheese made from heat-treated milk was used. For the case of *S. aureus*, criteria established por coagulase-positive *Sthapylococcus* were taken.

Twenty-five gram portions were obtained from each cheese sample, placed in a sterile masticator bag together with an appropriate volume (1/9) (w/v) of sterile 0.1% peptone water (Merck, Darmstadt, Germany), and homogenized in a masticator (AES, Combourg, France) for 1 min. After homogenization, samples were processed as follows.

E. coli

One milliliter of 1×10^{-1} to 1×10^{-3} dilutions of cheese extracts were processed on plates of Fluorocult® Agar prepared following manufacturers' directions (Merck). Once the agar had solidified, the plates were overlaid with 3–4 ml of melted Fluorocult and incubated at 44 °C for 24 h. After incubation, pink to red colonies exhibiting blue florescence after exposure to a 365 nm ultraviolet lamp (Vilbert Lourmat, Marne, France) were identified as *E. coli*. A maximum of three typical *E. coli* colonies isolated from each sample were picked, transferred onto Columbia agar supplemented with 5% sheep blood (BioMérieux, Marcy l`Etoile, France), and incubated at 44 °C for 24 h in order to obtain pure cultures. These pure cultures were first investigated by colony and cell morphology, Gram stain, oxidase and catalase activity, methyl red stain and indole production. Positive isolates preliminary identified as *E. coli* were confirmed by the API 20E identification system (BioMérieux).

S. aureus

For the case of *S. aureus*, 0.1 ml of 10^{-1} to 10^{-2} dilutions of cheese extracts were processed on plates of Baird Parker Agar prepared following manufacturers' directions (BioMérieux), and incubated at 37 °C for 48 h. Grey to black colonies with a white halo showing coagulase activity were isolated. A maximum of three typical *S. aureus* colonies isolated from each cheese sample were picked, transferred onto Columbia agar supplemented with 5% sheep blood (BioMérieux), and incubated at 37 °C for 48 h in order to obtain pure cultures. These pure cultures were first examined for colony and cell morphology, Gram stain, oxidase, and catalase activity. Positive isolates preliminary identified as *S. aureus* were confirmed by the API ID 32 STAPH identification system (BioMérieux).

Salmonella spp.

Buffered peptone water suspensions were incubated at 37 °C for 24 h. After this, 0.1 ml of incubated buffered peptone water broths were subcultured into 10 ml of Rappaport-Vassiliadis enrichment broth (Merck) and incubated at 42 °C for 24 h. The broths were subcultured onto xylose lysine desoxycholate agar (XLD) (Oxoid, Basingstoke, UK) and SM® ID 2 agar (BioMérieux). Black colonies in the XLD agar and purple colonies in SM ID 2 were considered to be *Salmonella* spp. A maximum of three typical *Salmonella* colonies isolated from each cheese sample were picked, transferred onto Columbia agar supplemented with 5% sheep blood (BioMérieux), and incubated at 37 °C for 48 h in order to obtain pure cultures. These pure cultures were examined for colony and cell morphology, Gram stain, oxidase and catalase activity. Positive isolates preliminary identified as *Salmonella* spp. were confirmed by the API 20 E identification system (BioMérieux).

L. monocytogenes

For *L. monocytogenes* identification, 0.2 ml of 10^{-1} to 10^{-2} dilutions of cheese extracts were processed on plates of Ottaviani and Agosti (ALOA) (AES) and incubated at 37 °C for 48 h for characteristic *Listeria* colonies. Blue/green colonies with an opaque halo were considered to be *L. monocytogenes*. Two typical *L. monocytogenes* colonies isolated from each cheese sample were picked, transferred onto Columbia agar supplemented with 5% sheep blood (BioMérieux), and incubated for 24 h at 37 °C in order to obtain pure cultures. Such pure cultures were first examined for their colony and cell morphology, Gram stain, oxidase and catalase activity, tumbling motility at 25 °C, and haemolysis on sheep blood agar. Positive isolates preliminary identified as *L. monocytogenes* were confirmed by the API listeria identification system (BioMérieux).

All isolates were stored at −80 °C in Maintenance Freeze Medium units (Oxoid) until antimicrobial susceptibility was tested.

Antimicrobial susceptibility testing of bacteria

Antimicrobial susceptibility testing was performed for a total of 287 isolates of *E. coli* and 281 isolates of *S. aureus*. Antimicrobial susceptibility testing was carried out by agar disk diffusion on Müeller-Hinton agar plates (Oxoid) according to the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) guidelines (NCCLS, 2002). Antimicrobial disks used for *E. coli* testing were as follows: ampicillin (10 μg), aztreonam (30 μg), cephalotin (30 μg), chloramphenicol (30 μg), doxycycline (30 μg), ciprofloxacin (5 μg), fosfomycin (200 μg), gentamicin (10 μg), nitrofurantoin (300 μg), streptomycin (10 μg) and sulfisoxazole (300 μg) (Oxoid). Antimicrobial disks used for *S. aureus* testing were: chloramphenicol (30 μg), clindamycin (2 μg), ciprofloxacin (5 μg), doxycycline (30 μg), erythromycin (15 μg), gentamicin (10 μg), penicillin (10 UI), oxacillin (1 μg), nitrofurantoin (300 μg), rifampin (5 μg) and sulfisoxazole (300 μg) (Oxoid).

Antimicrobial agents were selected in terms of their different structures and mechanisms of action. Antibiotic resistance breakpoints considered for *E. coli* and *S. aureus* were those recommended by the CLSI (NCCLS, 2002) for Enterobacteriaceae and *Staphylococcus* spp., respectively. *E. coli* ATCC 25922 and *S. aureus* ATCC 29213 were used as reference strains for this study. Isolates were classified as sensitive, intermediate (moderately sensitive) or resistant. Isolates exhibiting resistance to at least two of the antimicrobial agents tested were considered to be multi-resistant isolates.

Statistical analysis

The microbiological qualities of cheese made from raw conventional, pasteurized conventional or pasteurized organic milk were grouped into three different categories (satisfactory, borderline and unsatisfactory) prior to statistical analysis. The distributions of bacterial grade of acceptance as well as the distributions of resistant isolates and multi-resistance patterns were compared using the χ^2 test. The differences were considered to be significant when P was less than 0.05. All statistical analyses were performed using Statgraphics version 5.0.1 software (SAS Institute, North Carolina, USA).

Results

Applying the criteria established in European Regulation 2073/2005 (European Commission, 2005) (Table 1), 61.4% of the RCC samples were of satisfactory microbiological quality, 22.8% were of borderline quality and 15.8% were of unsatisfactory quality due to high levels of *S. aureus, E. coli* and/or *L. monocytogenes* (Table 2). *Salmonella* spp. was not detected in any examined RCC samples. For PCC samples, 71.6% were of satisfactory microbiological quality, 16.4% were of borderline and 11.9% were of unsatisfactory quality due to high levels of *S. aureus*, *E. coli*, *L. monocytogenes* and/or *Salmonella* spp. For POC samples, 65% were of satisfactory microbiological quality, 18.3% were borderline and 16.7% were of unsatisfactory quality due to high levels of *S. aureus* and/or *E. coli*. No *L. monocytogenes* and/or *Salmonella* spp. were detected in the POC samples analyzed. No significant differences in the microbiological general acceptance between the three types of cheese analyzed were seen. For *L. monocytogenes*, higher levels of unacceptability were observed for the case of RCC when compared to PCC ($P = 0.0334$) and POC ($P = 0.0138$) samples.

Regarding the antimicrobial resistance of isolates, different patterns were observed in the *E. coli* (Table 3) and *S. aureus* (Table 4) isolates isolated from RCC, PCC and POC samples. Significant differences in antimicrobial resistance were obtained in *E. coli* isolates derived from RCC with respect to isolates derived from PCC for three antimicrobials (ciprofloxacin, doxycicline and sulfisoxazole), and with respect to isolates derived from POC for six antimicrobials (ampicillin, ciprofloxacin, doxycycline, forfomycin, streptomycin and sulfisoxazole). Additionally, significant differences in antimicrobial resistance of *E. coli* isolates from PCC and POC were found for thee antimicrobials (ampicillin, doxycycline and streptomycin). For all antimicrobials tested, *E. coli* isolates obtained from POC showed similar or lower resistance levels than isolates from RCC and PCC, with the exception of fosfomycin, for which isolates obtained from POC showed higher resistance level than isolates obtained from RCC.

For *S. aureus* (Table 4), significant differences were seen in isolates from RCC and PCC samples only for the case of Rifampin. Nevertheless, significant differences in antimicrobial resistance were seen between isolates from RCC and POC samples for six antimicrobials (clindamicin, ciprofloxacin, gentamicin, penicillin, oxacillin and rifampin), and significant differences were also seen between isolates from PCC and POC for five antimicrobials (ciprofloxacin, gentamicin, penicillin, rifampin and sulfisoxazole). For all antimicrobials tested, *S. aureus* isolates obtained from POC showed similar or lower resistance levels than isolates from RCC and PCC, with the exceptions of gentamicin, for which isolates obtained from POC showed higher resistance level than isolates obtained both from RCC and PCC.

Nevertheless, despite the differences found in resistance rates between *E. coli* and *S. aureus* isolated from RCC, PCC and POC samples, no significant differences were found in the percentages of multiresistant isolates (Table 5), except for a higher percentage found in the *E. coli* isolated from RCC than for *E. coli* isolated from POC samples

Table 1. Classification of cheeses made from raw and pasteurized milk according to microbiological criteria established by European Regulation 2073/2005.

Tabla 1. Clasificación de los quesos fabricados a partir de leche cruda y pasteurizada de acuerdo a los criterios microbiológicos establecidos en el Reglamento Europeo 2073/2005.

	Microbiological quality (cfu g^{-1})		
Microorganism	Satisfactory	Borderline	Unsatisfactory
E. coli			
All cases	$<10^2$	10^2 to $<10^3$	$\geq 10^3$
*S. aureus**			
Raw or thermized milk	$<10^4$	10^4 to $<10^5$	$\geq 10^5$
Pasteurized milk	$<10^2$	10^2 to $<10^3$	$\geq 10^3$
L. monocytogenes			
All cases	Not detected	Detected 10^2	$\geq 10^2$
Salmonella spp.			
All cases	Not detected		Detected

$n = 5$ and $c = 2$ for all cases except for *L. monocytogenes* and *Salmonella* spp. ($c = 0$).
*Criteria established for coagulase-positive *staphylococci*.

$n = 5$ y $c = 2$ para todos los casos except *L. monocytogenes* y *Salmonella* spp. ($c = 0$).
*Criterios establecidos para *Staphylococcus* coagulasa-postivos.

Table 2. Microbiological acceptance of raw conventional cheese (RCC), pasteurized conventional cheese (PCC) and pasteurized organic cheese (POC) according to European Regulation 2073/2005.

Tabla 2. Aceptabilidad microbiológica de los quesos de leche cruda convencional (RCC), de leche cruda pasteurizada (PCC), y de leche pasteurizada ecológica (POC), según lo establecido en el Reglamento Europeo 2073/2005.

Cheese type (*n*)	Microorganism	Microbiological quality (*n*)		
		Satisfactory	Borderline	Unsatisfactory
RCC (57)	*E. coli*	42	11	4
	S. aureus	47	6	3
	Salmonella spp.	57	–	0
	L. monocytogenes	48	–	7
	Total cheese samples	35	13	9
PCC (67)	*E. coli*	58	6	3
	S. aureus	50	11	6
	Salmonella spp.	65	–	2
	L. monocytogenes	66	–	1
	Total cheese samples	48	11	8
POC (60)	*E. coli*	43	9	7
	S. aureus	50	3	8
	Salmonella spp.	60	–	0
	L. monocytogenes	60	–	0
	Total cheese samples	39	11	10

Significant differences were obtained in *L. monocytogenes* unacceptability between RCC-PCC ($P = 0.0334$) and RCC-POC ($P = 0.0138$).

Se encontraron diferencias significativas en la aceptabilidad de la contaminación por *L. monocytogenes* entre RCC y PCC ($P = 0{,}0334$) y entre RCC y POC ($P = 0{,}0138$).

Table 3. Number (Percentage) of *Escherichia coli* isolates obtained from raw conventional cheese (RCC), pasteurized conventional cheese (PCC), and pasteurized organic cheese (POC) that exhibited a sensitive (S), intermediate (I) or resistant (R) phenotype with respect to antimicrobial agents.

Tabla 3. Número (Porcentaje) de aislamientos de *Escherichia coli* obtenidos a partir de queso crudo convencional (RCC), queso pasteurizado convencional (POC) y queso pasteurizado ecológico (POC), que mostraron un fenotipo sensible (S), de sensibilidad moderada (I) o resistente (R) a los distintos agentes antimicrobianos.

Antimicrobial agent (μg)	RCC ($n = 105$)			PCC ($n = 88$)			POC ($n = 94$)		
	S	I	R	S	I	R	S	I	R
Ampicillin (10)	41 (39)	23 (21.9)	41 (39)[a]	31 (35.2)	27 (30.7)	30 (34.1)[a]	54 (57.4)	21 (22.3)	19 (20.2)[b]
Aztreonam (30)	100 (95.2)	4 (3.8)	1 (0.9)[a]	83 (94.3)	5 (5.7)	0 (0)[a]	88 (93.6)	6 (6.4)	0 (0)[a]
Cephalotin (30)	73 (69.5)	12 (11.4)	20 (19)[a]	63 (71.6)	4 (4.5)	21 (23.9)[a]	70 (74.5)	4 (4.3)	20 (21.3)[a]
Chloramphenicol (30)	100 (95.2)	3 (2.9)	2 (1.9)[a]	85 (96.6)	1 (1.1)	2 (2.2)[a]	91 (96.8)	3 (3.2)	0 (0)[a]
Ciprofloxacin (5)	86 (81.9)	14 (13.3)	5 (4.7)[a]	83 (94.3)	4 (4.5)	1 (1.1)[b]	93 (98.9)	0 (0)	1 (1.1)[b]
Doxycycline (30)	71 (67.6)	17 (16.2)	17 (16.2)[a]	42 (47.7)	37 (42)	9 (10.2)[b]	77 (81.9)	6 (6.4)	11 (11.7)[c]
Fosfomycin (200)	94 (89.5)	9 (8.6)	2 (1.9)[a]	76 (86.4)	12 (13.6)	0 (0)[a,b]	71 (75.5)	19 (20.2)	4 (4.3)[b]
Gentamicin (10)	86 (81.9)	15 (14.3)	4 (3.8)[a]	75 (85.2)	11 (12.5)	2 (2.3)[a]	86 (91.5)	8 (8.5)	0 (0)[a]
Nitrofurantoin (300)	90 (85.7)	8 (7.6)	7 (6.7)[a]	65 (73.9)	17 (19.3)	6 (6.8)[a]	71 (75.5)	17 (18.1)	6 (6.4)[a]
Streptomycin (10)	36 (34.3)	42 (40)	27 (25.7)[a]	18 (20.5)	45 (51.1)	25 (28.4)[a]	59 (62.8)	16 (17)	19 (20.2)[b]
Sulfisoxazole (300)	41 (39)	12 (11.4)	52 (49.5)[a]	60 (68.2)	7 (8)	21 (23.9)[b]	64 (68.1)	10 (10.6)	20 (21.3)[b]

[a,b,c]Values in the same row with different letters are significantly different.

[a,b,c]Los valores en la misma fila con letras diferentes presentan diferencias estadísticamente significativas.

($P = 0.017$). No statistically significant differences between the percentages of multi-resistant *S. aureus* isolates isolated from RCC, PCC and POC samples were seen.

Discussion

This study showed that the vast majority of retail Arzua-Ulloa type cheeses sold in Galicia were of satisfactory or borderline microbiological quality according to criteria established in European Regulation 2073/2005 (European Commission, 2005). However, in contrast to previous microbiological comparative studies regarding organic and conventional foodstuffs such as poultry (Bailey & Cosby 2005; Cui et al., 2005; Heuer, Pedersen, Andersen, & Madsen, 2001; Miranda et al., 2007, 2008a; Soonthornchaikul et al., 2006) or pork (Miranda et al., 2008b), no differences in the microbiological contamination of conventional and organic Arzúa-Ulloa cheeses were seen. Although it is reasonable to think that the pasteurization process should eliminate the vast majority of the bacterial population, many cheese-makers add raw milk to the cheese milk, considering it essential for good flavour due to the greater proteolysis

Table 4. Number (Percentage) of *Staphylococcus aureus* isolates obtained from raw conventional cheese (RCC), pasteurized conventional cheese (PCC) and pasteurized organic cheese (POC) that exhibited a sensitive (S), intermediate (I) or resistant (R) phenotype with respect to antimicrobial agents.

Tabla 4. Número (Porcentaje) de aislamientos de *Staphylococcus aureus* obtenidos a partir de queso crudo convencional (RCC), queso pasteurizado convencional (POC) y queso pasteurizado ecológico (POC), que mostraron un fenotipo sensible (S), de sensibilidad moderada (I) o resistente (R) a los distintos agentes antimicrobianos.

Antimicrobial agent (μg)	RCC (n = 98)			PCC (n = 92)			POC (n = 91)		
	S	I	R	S	I	R	S	I	R
Chloramphenicol (30)	86 (87.8)	6 (6.1)	6 (6.1)[a]	88 (95.7)	2 (2.2)	2 (2.2)[a]	84 (92.3)	4 (4.4)	3 (3.3)[a]
Clindamycin (2)	51 (52)	25 (25.5)	22 (22.4)[a]	60 (65.2)	7 (7.6)	25 (27.2)[b]	64 (70.3)	13 (14.3)	14 (15.4)[b]
Ciprofloxacin (5)	71 (72.4)	9 (9.2)	18 (18.4)[a]	68 (73.9)	9 (9.8)	15 (16.3)[a]	81 (89)	4 (4.4)	6 (6.6)[b]
Doxycycline (30)	64 (65.3)	23 (23.5)	11 (11.2)[a]	72 (78.3)	12 (13)	8 (8.7)[a]	59 (64.8)	22 (24.2)	10 (11)[a]
Erythromycin (15)	62 (63.3)	18 (18.4)	18 (18.4)[a]	58 (63)	14 (15.2)	20 (21.7)[a]	68 (74.7)	9 (9.9)	14 (15.4)[a]
Gentamicin (10)	87 (88.8)	9 (9.2)	2 (2)[a]	84 (91.3)	5 (5.4)	3 (3.3)[a]	71 (78)	11 (12.1)	9 (9.9)[b]
Penicillin (10 UI)	36 (36.7)	–	62 (63.3)[a]	41 (44.6)	–	51 (55.4)[a]	55 (60.4)	–	36 (39.6)[b]
Oxacillin (1)	36 (36.7)	15 (15.3)	47 (48)[a]	44 (47.8)	12 (13)	36 (39.1)[a]	64 (70.3)	9 (9.9)	18 (19.8)[b]
Nitrofurantoin (300)	84 (85.7)	9 (9.2)	5 (5.1)[a]	78 (84.8)	6 (6.5)	8 (8.7)[a]	83 (91.2)	4 (4.4)	4 (4.4)[a]
Rifampin (5)	81 (82.7)	13 (13.3)	4 (4.1)[a]	64 (69.6)	21 (22.8)	7 (7.6)[a]	86 (94.5)	3 (3.3)	2 (2.2)[b]
Sulfisoxazole (300)	50 (51)	11 (11.2)	37 (37.8)[a,b]	56 (60.9)	3 (3.3)	33 (35.9)[a]	45 (49.5)	13 (14.3)	33 (36.3)[b]

[a,b,c]Values in the same row with different letters are significantly different.

[a,b,c]Los valores en la misma fila con letras diferentes presentan diferencias estadísticamente significativas.

Table 5. Resistance patterns in *Escherichia coli* and *Staphylococcus aureus* isolates obtained from isolated from raw conventional cheese (RCC), pasteurized conventional cheese (PCC), and pasteurized organic cheese (POC).

Tabla 5. Patrones de multiresistencia en aislamientos de *Escherichia coli* y *Staphylococcus aureus* obtenidos a partir de queso crudo convencional (RCC), queso pasteurizado convencional (PCC), y queso pasteurizado ecológico (POC).

Number of antimicrobials	*Escherichia coli*			*Staphylococus auerus*		
	RCC (n = 105) No. (%)	PCC (n = 88) No. (%)	POC (n = 94) No. (%)	RCC (n = 98) No. (%)	PCC (n = 92) No. (%)	POC (n = 91) No. (%)
0	30 (28.6)	29 (33)	40 (42.6)	9 (9.2)	10 (10.9)	20 (22)
1	24 (22.9)	24 (27.3)	31 (33)	27 (27.6)	21 (22.8)	23 (25.3)
2	17 (16.2)	21 (23.9)	9 (9.6)	26 (26.5)	27 (29.3)	28 (30.8)
3	23 (21.9)	9 (10.2)	10 (10.6)	16 (16.3)	15 (16.3)	11 (12.1)
4	9 (8.6)	4 (4.5)	2 (2.1)	11 (11.2)	13 (14.1)	6 (6.6)
≥5	2 (1.9)	1 (1.3)	2 (2.1)	9 (9.2)	6 (6.5)	3 (3.3)
Multi-resistant isolates (%)	51 (48.6)	35 (39.8)	23 (24.5)	62 (63.3)	61 (66.3)	48 (52.7)

Significantly differences were found between *E. coli* isolated from RCC and POC (P = 0.017).

Diferencias significativas fueron encontradas entre *E. coli* aislados a partir de RCC y POC (P = 0,017).

and lipolysis by the raw milk microflora in the cheese (Little et al., 2008). Thus, the fact that the only difference in the microbiological acceptance (higher unsatisfactory levels of *L. monocytogenes* in RCC than in PCC or POC samples) affects conventional and organic pasteurized cheeses equally shows that the microbiological quality of cheese is more influenced by factors such as heat treatment or hygiene during manufacture, packaging and handling, than by the type of milk used for cheese manufacturing.

Regarding the antimicrobial resistance, variable rates of antimicrobial resistance were reported for *E. coli* and *S. aureus* isolates obtained from conventional milk, milk products and dairy cows. The antimicrobial resistance levels obtained in the present study are largely in agreement with those reported by others authors (Busato et al., 2000; DeFrancesco, Cobbold, Rice, Besser, & Hancock, 2004; Srinivasan et al., 2007; Tikofsky et al., 2003)

The relationship between the use of antimicrobial agents in animal farming and the presence of antimicrobial resistant bacteria in the food products obtained from these animals has previously been reported by other authors (Aarestrup, 2000; Asai et al., 2005; Van den Boogard, London, Driessen, & Stobberingh, 2001). Antimicrobials (mostly β-lactams alone and in combination with other antibiotics) are regularly used in conventional dairy herds. However, in the case of organic herds, the use of antimicrobials is seriously restricted by organic farming regulations (Busato et al., 2000; Melchior, Fink-Gremmels, & Gaastra, 2007).Taking into account these serious restrictions, it is expected that the antimicrobial resistance of bacteria isolated from organic dairy products should be lower than the antimicrobial resistance of isolates from conventional products, especially for bacteria such as *Escherichia coli*, which has been described as a very useful biomarker to evaluate the development of antimicrobial resistance (Von Baum & Marre, 2005) or *S. aureus*, one of the major causes of mastitis in dairy cattle (Melchior et al., 2007), which often requires antibiotic treatment (Sandgren et al., 2008).

In this sense, both *E. coli* and *S. aureus* isolated from RCC and PCC showed higher levels than those obtained from POC samples for antimicrobial agents commonly used in dairy herd medicine such as ampicillin, doxycycline, and streptomycin (in the case of *E. coli*) and ciprofloxacin, penicillin, oxacillin and rifampin (in the case of *S. aureus*). The fact that no significant differences were observed for antimicrobial agents banned for veterinary medicine in the European Union, such as chloranphenicol and nitrofurantoin, provides additional support to the hypothesis that organic farming methods contribute to the reduction of the development and spread of antimicrobial resistance.

However, other results do not support this hypothesis. Significant differences were obtained between isolates from RCC and PCC samples for some antimicrobials (ciprofloxacin, doxycycline and sulfisoxazole for the case of *E. coli* and clindamycin for the case of *S. aureus*). Significantly higher levels of antimicrobial resistance were obtained in isolates from POC samples rather than conventional products, as the higher resistance of *E. coli* isolated from POC than RCC for fosfomycin or the higher gentamicin resistance found in *S. aureus* isolates from POC samples than those found for isolates obtained from RCC or PCC samples. These differences cannot be attributed to the frequency of antimicrobial use at the farm level and seem to suggest an important influence of external factors, such as contamination by the environment or handlers. The absence of significant differences of multiresistance rates observed for all cases but *E. coli* between RCC and POC products indicates that there is not enough evidence of a lower antimicrobial use in organic dairy herds.

Although organic products deserve higher prices in the market than conventional ones, the sales of organic foods has increased in the recent years (Magkos et al., 2006) due to consumers' belief that organic food is substantially healthier and safer than conventional food. Nevertheless, the results obtained in the present work indicate that the microbiological contamination of organic and conventional Arzua-Ulloa cheeses is similar for products processed in the same conditions. Although lower levels of antimicrobial resistance of *E. coli* and *S. aureus* isolated from organic products than from conventional ones were found for antimicrobials commonly used in bovine medicine, for the case of other antimicrobials these differences were ambiguous or even showed higher levels in isolates from organic products. Thus, although further studies are needed, from a microbiological point of view, there is not enough evidence justifying the consumers' perceptions about the better microbiological healthiness of organic Arzua-Ulloa cheeses when compared to conventional cheese.

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References

Aarestrup, F.M. (2000). Occurrence, selection and spread of resistance to antimicrobial agents used for growth promotion for food animals in Denmark. *APMIS*, *108*(Suppl 101), 5–48.

Asai, T., Kojima, A., Harada, K., Ishihara, K., Takahashi, T., & Tamura, Y. (2005). Correlation between the usage volume of veterinary therapeutic antimicrobials and resistance in *Escherichia coli* isolated from the faeces of food-producing animals in Japan. *Japanese Journal of Infectious Disesases*, *58*, 369–372.

Bailey, J.S., & Cosby, D.E. (2005). *Salmonella* prevalence in free-range and certified organic chikens. *Journal of Food Protection*, *68*, 2451–2453.

Busato, A., Traschel, P., Schällibaun, M., & Blum, J.W. (2000). Udder health and risk factors for subclinical mastitis in organic dairy farms in Switzerland. *Preventive Veterinary Medicine*, *44*, 205–220.

Cui, S., Ge, B., Zheng, J., & Jianghong, M. (2005). Prevalence and antimicrobial resistance of *Campylobacter* spp. and *Salmonella* serovars in organic chickens in Maryland retail stores. *Applied and Environmental Microbiology*, *71*, 4108–4111.

De Buyser, M.L., Dufour, B., Maire, M., & Lafarge, V. (2001). Implication of milk and milk products in food-borne diseases in France and in different industrialised countries. *International Journal of Food Microbiology*, *67*, 1–17.

DeFrancesco, K., Cobbold, R.N., Rice, D.H., Besser, T., & Hancock, D.D. (2004). Antimicrobial resistance of commensal *Escherichia coli* from dairy cattle associated with recent multi-resistant salmonellosis outbreaks. *Veterinary Microbiology*, *98*, 55–61.

European Commision. (2005). Commission Regulation (EC) No. 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. *Official Journal of European Union, L*, *338*, 1–26.

Guinot-Thomas, P., Jonderville, C., & Laurent, F. (1991). Comparison of milk from farms with biological, conventional and transitional feeding. *Milchwissenchaft*, *46*, 779–782.

Heuer, O.E., Pedersen, K., Andersen, J.S., & Madsen, M. (2001). Prevalence and antimicrobial susceptibility of thermophilic *Campylobacter* in organic and conventional broiler flocks. *Letters in Applied Microbiology*, *33*, 269–274.

Litle, C.L., Rhoades, J.R., Sagoo, S.K., Harris, J., Greenwood, M., Mithani, V., Grant, K., & McLauchlin, J. (2008). Microbiological quality of retail cheeses made from raw, thermized or pasteurized milk in the UK. *Food Microbiology*, *25*, 304–312.

Lund, P. (1991). Characterization of alternatively produced milk. *Milchwissenschaft*, *46*, 166–169.

Luukkonen, J., Kemppinen, A., Kärki, M., Laitinen, H., Mäki, M., Sivelä, S., Taimisto, A.M., & Ryhänen, E.L. (2005). The effect of a protective culture and exclusion of nitrate on the survival of enterohemorrhagic *E. coli* and *Listeria* in Edam Cheese made from Finnish organic milk. *International Dairy Journal*, *15*, 449–457.

Magkos, F., Arvaniti, F., & Zampelas, A. (2006). Organic food: buying more safety or just peace of mind? A critical review of the literature. *Critical Reviews in Food Science and Nutrition*, *46*, 23–56.

Melchior, M.B., Fink-Gremmels, J., & Gaastra, W. (2007). Extended antimicrobial susceptibility assay for *Staphylococcus aureus* isolates from bovine mastitis growing in biofilms. *Veterinary Microbiology*, *125*, 141–149.

Miranda, J.M., Guarddon, M., Mondragón, A., Vázquez, B.I., Fente, C.A., Cepeda, A., & Franco, C.M. (2007). Antimicrobial resistance in *Enterococcus* spp. strains isolated from organic chicken, conventional chicken and turkey meat: a comparative survey. *Journal of Food Protection*, *70*, 1021–1024.

Miranda, J.M., Guarddon, M., Vázquez, B.I., Fente, C.A., Barros-Velázquez, J., Cepeda, A., & Franco, C.M. (2008a). Antimicrobial resistance in Enterobacteriaceae strains isolated from organic chicken, conventional chicken and conventional turkey meat: a comparative survey. *Food Control*, *19*, 412–416.

Miranda, J.M., Vázquez, B.I., Fente, C.A., Barros-Velázquez, J., Cepeda, A., & Franco, C.M. (2008b). Antimicrobial resistance in *Escherichia coli* strains isolated from organic and conventional pork meat: a comparative survey. *European Food Research and Technology*, *226*, 371–375.

National Committee for Clinical Laboratory Standards (NCCLS). (2002). *Performance standards for antimicrobial susceptibility testing; 12th informational supplement*, M100-S12. Wayne, PA: NCCLS.

Phillips, I., Casewell, M., Cox, T., De Groot, B., Friis, C., Jones, R., Nightingale, C., Preston, R., & Waddell, J. (2004). Does the use of antimicrobials in food animals pose a risk to human health? A critical review of published data. *Journal of Antimicrobial Chemotherapy*, *53*, 28–52.

Rosati, A., & Aumaitre, A. (2004). Organic dairy farmig in Europe. *Livestock Production Science*, *90*, 41–51.

Sandgren, C.H., Waller, K.P., & Emanuelson, U. (2008). Therapeutic effects of systemic or intramammary antimicrobial treatment of bovine subclinical mastitis during lactation. *The Veterinary Journal*, *175*, 108–117.

Sato, K., Bartlett, P.C., Erskine, R.J., & Kaneene, J.B. (2005). A comparison of production and management between Wisconsin organic and conventional herds. *Livestock Production Science*, *93*, 105–115.

Soonthornchaikul, N., Garelick, H., Jones, H., Jacobs, J., Ball, D., & Choudhury, M. (2006). Resistance to three antimicrobial agents of *Campylobacter* isolated from organically- and intensively-reared chickens purchased from retail outlets. *International Journal of Antimicrobial Agents*, *27*, 125–130.

Srinivasan, V., Gillespie, B.E., Lewis, M.J., Nguyen, L.T., Headrick, S.I., Schuckken, Y.H., & Oliver, S.P. (2007). Phenotypic and genotypic antimicrobial resistance patterns of *Escherichia coli* isolated from dairy cows with mastitis. *Veterinary Microbiology*, *124*, 319–328.

Tikofsky, L.L., Barlow, J.W., Santiesteban, C., & Schukken, Y.H. (2003). A comparison of antimicrobial susceptibility patterns for *Staphylococcus aureus* in organic and conventional dairy herds. *Microbial Drug Resistance*, *9*, 39–45.

Van den Bogaard, A.E., London, N., Driessen, C., & Stobberingh, E.E. (2001). Antibiotic resistance of faecal *Escherichia coli* in poultry, poultry farmers and poultry slaughterers. *Journal of Antimicrobial Chemotherapy*, *47*, 763–771.

Von Baum, H., & Marre, R. (2005). Antimicrobial resistance of *Escherichia coli* and therapeutic implications. *International Journal of Medical Microbiology*, *295*, 503–511.