Comparison of the epidemiological behavior of mastitis pathogens by applying time-series analysis in results of milk samples submitted for microbiological examination.

Fernández G[a]., Barreal M.L.[b], Pombo M.B.[b], Ginzo-Villamayor M.J.[c], González-Manteiga W.[c], Prieto A.[a], Lago N.[a,*], González-Palencia J.[b]

[a]INVESAGA. *Investigación en Sanidad Animal de Galicia. Universidad de Santiago de Compostela. Spain.*

[b]LIGAL. *Laboratorio Interprofesional Gallego de Leche. Spain.*

[c]*Departamento de estadística e investigación operativa. Universidad de Santiago de Compostela. Spain*

*Corresponding author. Telf.: +34 66299999322127. *E-mail address*: noelia.lago@rai.usc.es

ABSTRACT

The objective of this study is to examine and compare the trends of mastitis pathogens in quarter milk samples (n=240,232) submitted for microbiological examination at the Milk Analysis Laboratory (L.I.G.A.L.) at Galicia, Spain from June 2005 to September 2011. Autoregressive Integrated Moving Average (ARIMA) models and multivariate statistical techniques such as Cluster Analysis were used in order to detect seasonal trends and similarities between the series trends and to classify mastitis pathogens into relatively homogeneous groups.

The decrease of bulk milk somatic cell counts achieved by the mastitis control program, developed in recent years in this region, is the result of the decrease in IMI caused by a limited number of mastitis pathogens. The obtained results reflect a greater complexity in the behavior of mastitis pathogens, unlike the traditional classification into contagious or environmental. *Staphylococcus aureus* showed a trend similar to *Streptococcus dysgalactiae*, a mastitis pathogen can behave in both a contagious and an environmental manner. Among the traditionally considered environmental mastitis pathogens, *Strep. uberis* showed a different behavior to *Escherichia coli* and *Klebsiella pneumoniae.* Coagulase-negative staphylococci (CNS) species and Streptococcus other than *Strep. agalactiae* showed differences in the trend model. Time-series analysis and multivariate statistical techniques, such as Cluster Analysis, could be powerful tools to assess the isolation trend of mastitis pathogens because of their ability to cope with stochastic dependence of consecutive data. Furthermore, they could be used to identify the epidemiological behavior of mastitis pathogens using the results of milk samples submitted for routine microbiological examination, by classifying them into relatively homogeneous groups.

(**Key words:** dairy cattle, mastitis, pathogens, trends, epidemiology, time series)

INTRODUCTION

More than 130 different organisms can cause bovine mastitis (Watts, 1988). However, intra-mammary infections (IMI) are mostly caused by a much smaller range of pathogens. Traditionally, mastitis-causing pathogens have been classified as either `contagious´ or `environmental´, depending on their primary behavior and their mode of transmission (Smith and Hogan, 1995). Contatious pathogens include *Streptococcus agalactiae*, *Staphylococcus aureus* and *Corynebacterium* spp. and environmental pathogens include mainly coliforms and *Streptococcus uberis*.

The introduction of mastitis control strategies based on the Five-Point Plan of Mastitis Control resulted in a rapid progress to control both clinical and subclinical mastitis and has led to a massive reduction in bulk milk somatic cell counts worldwide (Bradley, 2002). Nevertheless, the observed decrease in the prevalence of contagious mastitis pathogens has been accompanied by an increase in the prevalence of environmental pathogens (Myllys et al., 1998; Bradley, 2002; Hogan and Smith, 2003), CNS (Sampimon et al., 2009), and uncommon pathogens such as yeasts or *Prototheca* spp. (Krukowski et al., 2006; Jagielski et al., 2011).

It has always been tried to classify mastitis pathogens as either environmental or contagious. However, some of them have not been clearly included in one of these groups. *Strep. dysgalactiae* has been described as a contagious pathogen and also as an environmental one (Smith and Hogan, 1995) and this fact remains a topic of discussion for CNS (Piessens et al., 2012). A potential drawback of many studies is the classification of CNS as a homogeneous group, as they have yielded conflicting results. CNS have been traditionally considered normal skin microbiota which can cause mastitis as opportunistic bacteria (Devriese and De Keyser, 1980). However, there are studies indicating that not all species fit well into this definition (Taponen et al., 2008) and that there are epidemiological and pathological differences between species of CNS (Zadoks et al., 2011b).

In recent decades, microbiological studies and those based on molecular biology have shown differences in the epidemiological behavior between mastitis pathogens, but also between strains within a species (Zadoks et al., 2003; Haveri et al., 2008). These findings have led to the conclusion that the traditional classification into environmental and contagious pathogens can't be so clearly applied to some mastitis pathogens (Bradley, 2002).

Usual statistical methods studies of results of milk samples submitted for microbiological examination assume that the observed data are realizations (certain value that is assigned to the random variable) of independent random variables (Makovec and Ruegg, 2003). However, any variable measured over time, as sequenced observations on isolation proportion, is not independent and is potentially influenced by previous observations (autocorrelation) and applying simple regression analysis would be inappropriate (Box and Jenkins, 1976). Since the early 1970s, time series methods, in particular ARIMA models (autoregressive integrated moving average models) which have the ability to cope with stochastic dependence of consecutive data, have become well established in such fields as industry, economics and epidemiology (Zeger et al., 2006).

A better understanding of the epidemiology of mastitis pathogens is useful for the dairy industry to prevent infections by implementation of specific control measures (Piessens et al., 2011). The objective of this study is to examine and compare the trends of mastitis pathogens in quarter milk samples (n=240,232) submitted for microbiological examination at the Galician Milk Analysis Laboratory (L.I.G.A.L.) from June 2005 to

September 2011 through the ARIMA methodology and multivariate statistical techniques such as Cluster Analysis, in order to detect seasonal trends and similarities between the series trends and to classify mastitis pathogens into relatively homogeneous groups.

MATERIALS AND METHODS

Study area

Galicia (NW Spain) is the largest livestock rearing region in Spain, accounting for 53% of Spain´s dairy farms and approximately 350,000 dairy cows older than two years. It is responsible for 35% of the milk in Spain, constituting about 1.7% of the milk produced in the European Union. During the period of this study the geometric mean for bulk milk somatic cell counts there has been decrease from 281,000 to 228,000 cell/ml (LIGAL's own data). Dairy farms are mainly located at 650 m elevation with low to moderate precipitation (<1500 mm/y) and moderate temperatures ($\bar{x}$ = 12.3 ◦C). The highest rainfall is recorded in November–December. The highest temperatures are observed in summer (June-August) and the lowest in winter (December-February).

Collection and processing of samples

All quarter milk samples submitted to LIGAL for microbiological examination from June 2005 to September 2011 were included in the study (76 months). They were collected by veterinarians and farm personnel and submitted to the laboratory when clinical mastitis was detected or when milk tested positive for California Mastitis Tests in surveillance programs for the whole herd monitoring.

Milk samples were analyzed using standard microbiologic methods according to National Mastitis Council (Hogan et al., 1999). A milk sample was considered culture-positive when at least one colony-forming unit (CFU) of *Strep. agalactiae* or *Staph. aureus* or five or more CFU of other microorganism were isolated. In cases with no growth or if none of the above definitions were met, the samples were considered without isolation. In case of double isolation only major pathogens were considered (defined as other than CNS and *Corynebacterium* spp.). Primary culture plates containing more than two colony types were considered to be contaminated, unless *Staph. aureus* or *Strep. agalactiae* were isolated. The total number of bacteriological results included mastitis pathogens isolated from pure culture or from milk samples that yielded two mastitis pathogens.

Preliminary identification of isolates was based on colony morphology, hemolytic patterns, Gram stain and catalase. Furthermore, isolates were identified to species level using ViITEK 2 system (bioMérieux, Hazelwood, MO, USA) with Vitek-2 cards: GP-test kit (Gram-positive cocci), GN-test kit (Gram-negative) and YST test kit (Yeast and *Prothoteca* spp.) The procedures recommended by the manufacturer were strictly followed. The bacterial identification was regarded as acceptable when the confidence level of the analysis was >85%.

Statistical Analysis

The time series analysis was performed on mastitis pathogens with at least 0.3% proportion of isolation over total samples. Statistical analysis included isolates identified to species level, except in the case of *Lactotoccus* spp. and yeast which were considered as a group debido a la baja frecuencia de aislados identificados a nivel de especie. The series values were the proportion of each mastitis pathogen or those results related to the number of samples from each month.

The steps carried out in the statistical analysis were as follows:

Step 1: The ARIMA methodology (Box and Jenkins, 1976) was used for each mastitis pathogen series. Decomposition methods were used to identify and isolate each of the variational components that were present in the series (trend, seasonality, heteroskedasticity, etc.). Stationarity was analyzed and once the time series was "stabilized" using appropriate transformations, a study of the presence of regularities in the series was made in order to identify a possible ARIMA model. Autocorrelation functions (ACFs) and partial autocorrelation functions (PACFs) were identified and examining to see which of the potential three patterns were present in the data. After selecting the appropriate ARIMA model, its coefficients were estimated and, finally, a diagnosis was carried out. The residual~~s~~ were analyzed in order to prove whether the model that fitted our data was adequate. Ljung-Box test, Shapiro-Wilk and Kolmogorov-Smirnov tests (Shumway and Stoffer, 2006) were used to check independence and normality of residuals (p-value > 0.05 was considered, accepting the hypothesis of independence or normality).

Step 2: Each time series, Y_t, was decomposed into two parts, trend part and error part, $Y_t - m(t) = \varepsilon_t$, where $m(t)$ represents the trend and ε_t the error. For calculating the error, the seasonal and irregular parts of the time t were considered. Once the two parts of the series were separated, trend and error, the ARIMA methodology was applied to the random part. Consider two time series Y_1, Y_2; the distance between the two series was defined as (Vilar-Fernández and González-Manteiga, 2004):

$$d(Y_1, Y_2) = \int (m_1 - m_2)^2 \omega(t) dt + d(\theta_1, \theta_2) \quad (0.1),$$

where m_1, m_2 are the trends in each series, θ_1, θ_2 the set of parameters of the ARIMA model for the random part and $\omega(t)$ a measure of weight. In this case, $\omega(t) = 1$, all time series have the same weight. As the ARIMA models for the random part were practically identical, in order to determine the distance between each mastitis pathogen series, the following expression was used to calculate the distance between trends:

$$d(m_1, m_2) = \int (m_1 - m_2)^2 \omega(t) dt \quad (0.2)$$

The expression (0.2) can be approximate as follows:

$$d(m_1, m_2) = \int (m_1 - m_2)^2 \omega(t) dt \approx \frac{1}{n} \sum_{i=1}^{n} \left(m_1 - m_2\right)^2 \omega(t) dt \quad (0.3)$$

Step 3: Once this distance was defined, a multivariate analysis technique was applied (cluster) in order to obtain homogeneous groups, that is, those constructed so that the elements in each group are similar. Before applying the distance, the trend data have been standardized to mean 0 and variances 1.

Step 4: A dendrogram was made. For comparing the results the k-means classification method was applied. To determine the number of clusters, the PAM algorithm (Partitioning Around Medoids) was used. The methods used to confirm the number of clusters and calculate the dendrogram were: Complete Method or Complete Linkage Method, Single Linkage Clustering, Average Method and Method of Ward or Method Sum of Squares (Everitt, 2005).

The statistical software used was R, version R-2.15.0, and packages were timeSeries (Wuertz and Chalabi, 2012) and TSA (Chan and Ripley, 2012) for time series analysis, forecast for ARIMA methodology (Hyndam., 2012) and mclust for cluster analysis (Fraley et al., 2012).

RESULTS

The submitted milk samples (n=240,232) were characterized as no growth (n=30,561), contaminated (n=32,852), or containing bacterial pathogens (n=176,819). The total number of bacteriological results (n=178,673) included mastitis pathogens isolated from pure culture (n=174,965) and mastitis pathogens (n=3,708) isolated from milk samples (n=1,854) that yielded two mastitis pathogens. The proportion of double isolation samples was 0.8% (0.2-1.7% range).

The proportion results of studied mastitis pathogens (which represented 88.9% of total isolates), no growth and contaminated samples from total samples, and total pathogens are described in table 1. 5.4 % of catalase-positive and Gram-positive cocci, 1.9% of *Streptococcus* spp. other than *Strep. agalactiae*, 2,2 % of *Enterococcus* spp. and *Lactococcus* spp. and 1.3% of total Gram-negative could not be identified to the species level.

Table 1 shows ARIMA trend model and the highest and lowest monthly proportions of each mastitis pathogens. *Strep. uberis*, *Strep. dysgalactiae*, *Staph. aureus*, *Staph. xylosus*, *Staph. sciuri*, *S. marcescens*, *Corynebacterium* spp. and yeast showed seasonal trends.

The monthly trends of standardized isolation proportion among total samples of mastitis pathogens are showed in Fig. 1.

Both with the PAM (Partioning Around Medoids) algorithm and the K-means algorithm was obtained that the appropriate cluster number was 4. The respective cophenetic correlation coefficients used in the different methods to calculate the dendrogram were: 0.737, 0.691, 0.794 and 0.688 for the complete, single, average and ward methods, respectively. The complete method was selected because it had a coefficient similar to the coefficient of the average method, and the results had a better interpretation in the dendrogram. The sizes of the obtained clusters were 10, 3, 3 and 4 (Fig. 2).

DISCUSSION

This is the first study (to our knowledge) that evaluates and compares the results of milk samples submitted to diagnostic laboratory through the ARIMA methodology and multivariate statistical techniques such as Cluster Analysis. The proportion of isolation of a pathogen is influenced by the prevalence of IMI caused by each pathogen in the population. The prevalence of different mastitis pathogens depends on the implementation of mastitis control programs (Bradley, 2002), facilities and herd management practices (housing and production system, etc.) and sampled population (parity, stage of lactation, clinical or subclinical mastitis etc.) (Makovec and Ruegg, 2003). A potential drawback of trend studies of results of milk samples submitted for microbiological examination is that the proportion of isolation of a pathogen can also be influenced by the selection of samples within the herd and the herds of origin and it may vary over time (Makovec and Ruegg, 2003). Nevertheless, two mastitis pathogens with the same epidemiological behavior (with the same response to these variations) will have a similar isolation trend and two mastitis pathogens with the same epidemiological behavior will not have a different trend. Therefore, ARIMA methodology and multivariate statistical techniques such as Cluster Analysis, allow an adequate comparison of isolation trends of mastitis pathogens regarding those types of data. However, in the interpretation of results must also be borne in mind that it is possible that two mastitis pathogens with different epidemiological behavior may have a similar isolation trend as a result of different causes.

Genotyping is superior to phenotyping in the identification of mastitis pathogens and this fact is particularly important in the case of the CNS (Ruegg, 2009). However, the genotyping identification is not available to most routine diagnostic and research laboratories, molecular studies are based on a limited number of isolates and herds, and the species distribution of CNS and even genotypes within species vary from one herd to another (Gillespie et al., 2009; Piessens et al., 2011; Supré et al., 2011), so that the information provided by them has not permitted a clear epidemiological classification of the CNS (Piessens et al., 2012). For this reason many epidemiological studies have used phenotypic methods for the identification of CNS (Sawant et al 2009; Gillespie et al., 2009; Sampimon et al., 2009; Nam et al., 2009). The consistent use of a phenotypic identification system containing an adequate set of species of veterinary importance in the diagnostic algorithm should be sufficiently precise for mastitis control programs and many research needs (Ruegg 2009). Vitek 2 has improved its accuracy rates in identifying species, compared with previous versions of this automated system. Studies on the identification of staphylococci described a high rate of correct identification with Vitek 2, compared to molecular methods (Delmas et al., 2008; Chatzigeorgiou et al., 2011).

Strep. agalactiae, *S. epidermidis* and *C. bovis* showed similar trends over time, with strong decreases in the proportion of isolation. These findings are in agreement with the fact that, *Strep. agalactiae* and *C. bovis* are considered contagious pathogens and in areas where they have carried out control programs for years, they have reported a decreasing prevalence (Myllys et al., 1998; Andersen et al., 2003; Bradley et al., 2007; Piepers et al., 2007). *Staph. epidermidis,* in a way similar to *Strep. agalactiae,* are relatively uncommon on bovine skin and in the environment (Pissens et al., 2011). Although cow-to-cow transmission from infected udders cannot be ruled out as a means of spreading (Piessens et al., 2012), it was hypothesized that *Staph. epidermidis* might be introduced in a dairy herd via human sources, in particular the milkers' hands (Thorberg et al., 2006). It is expected a high efficiency from the measures related to milking hygiene in reducing the frequency of IMI caused by this mastitis pathogen. Our results indicate a greater influence of seasonal conditions on IMI by *Corynebacterium* spp. than on IMI by two other mastitis pathogens, because it was the only one of three that presented a seasonal trend.

Particularly noteworthy are the results observed for *Serratia marcescens* because it was in the same cluster of contagious pathogens such as *Strep. agalactiae*, and it was the only coliform that showed a seasonal trend, which suggests that its epidemiological behavior is different from the other coliforms studied. This mastitis pathogen has been seldom studied, but it is known that IMI by *S. marcescens* develop into clinical diseases less frequently, and clinical cases tend to be less severe than other mastitis caused by gram-negative bacteria (Todhunter et al., 1991).

Among the mastitis pathogens classically considered contagious, *Staph. aureus* is the only one with a proportion of isolation that did not strongly decrease over time. Although there is a decrease in prevalence in those areas where control programs have been carried out for years (Sommerhäuser et al., 2003), *Staph. aureus* continues to be a major cause of subclinical mastitis over the world (Østeras et al., 2006; Tenhagen et al., 2006, Piepers, 2007; Capurro, et al., 2010). The commonly used explanation for the disappointing results obtained from control efforts include more complex epidemiology than a typical contagious pathogen such as *Strep. agalactiae*, because several extra-mammary sources of *Staph. aureus* are known (Matos et al., 1991; Capurro et al., 2010). Molecular studies have shown that strains of *Staph. aureus* with an epidemiological behavior that corresponds to an environmental pathogen can coexist with contagious strains within the same population (Sommerhäuser et al., 2003; Haveri et al., 2008; Capurro et al., 2010). In this study the proportion of isolates of *Staph. aureus* in total samples (11.28) was lower than 15.3% described in 1997 for the same area (Marco et al., 1998). A possible explanation for this fact is a lower efficacy of the mastitis control program over time in relation to the IMI caused by *Staph. aureus*, due to the increase of the relative frequency of IMI caused by environmental strains. In this study, *Staph. aureus* shows a trend over time (same cluster) and a seasonal trend (with a higher proportion of isolation in summer) similar to those of *Strep. dysgalactiae*, a mastitis pathogen that may have both environmental and contagious behavior (Smith and Hogan, 1995). This fact seems to support the hypothesis of coexistence in the population of environmental and contagious *Staph. aureus* strains.

K. oxytoca, K. pneumoniae and *E. coli* were in the same cluster. While in *E. coli* (Nemeth et al., 1994) and *K. pneumoniae* (Munoz et al., 2006) fecal shedding contributes to the exposure and incidence of mastitis, *K. oxytoca* may have other possible sources of infection (Zadoks et al., 2011a).

Strep. uberis, CNS other than *Staph. epidermidis*, Yeast and *Prototheca spp.* were included in other cluster. These results seem to be consistent with the difficulty of classifying these mastitis pathogens into traditional groups (contagious or environmental). It is worth mentioning the results observed in *Strep. uberis*, which presented a series trend (cluster) different from that of coliforms, despite that all of them are classically considered environmental pathogens. Some reasons could explain these differences. In the case of *Strep. uberis* there is evidence of the existence of strains with both types of epidemiological behavior (Zadoks et al., 2003), there are persistent IMI (Zadoks et al., 2003; Pullinger et al., 2007) and cases of poor response to antibiotic treatment (Milne et al., 2005). Those circumstances can increase the subclinical mastitis cases in dairy herds with SCC lower than 400.000 cells./ml, and clinical cases may represent a small proportion of IMI (Zadoks et al 2003). In contrast, IMI caused by *E. coli* and *K. pneumoniae* usually exhibit clinical symptoms, being short-term processes which end with the death of either the host or the pathogen (Hogan and Smith, 2003). Furthermore, *Strep. uberis* was the only one of these traditionally considered environmental mastitis pathogens that showed a seasonal trend, with a higher proportion of isolation in autumn. It has been often reported an increased incidence of IMI by

Strep. uberis in seasons with high rainfall and humidity and low temperatures (Barkema et al., 1999). However, dry summers in NW Spain are not favorable conditions for the growth of gram-negative bacteria (hot and humid weather), which have been considered, in other studies, to explain an increased exposure in this season and with these pathogens (Makovec and Ruegg 2003).

Two groups of CNS species were showed in the same cluster. Within one group were included three CNS species: *Staph. simulans*, *Staph. haemolyticus* and *Staph. warneri*. These CNS have been associated with a transient IMI (Supré et al., 2011) and an environmental origin. In a recent study, *Staph. simulans* and *Staph. haemolyticus* were regularly isolated from the environment samples (Piessens et al., 2011). In both of them it has been observed a genetic diversity in strains from the same herd, thus suggesting the existence of multiple sources other than the contagious nature of the pathogen (Aarestrup et al., 1999; Piessens et al., 2012).

Within the other group of the same cluster three CNS species were included: *Staph. chromogenes*, *Staph. sciuri* and *Staph. xylosus*. *Staph. chromogenes* and *Staph. xylosus* are some of the CNS with a higher frequency of persistent IMI (Supré et al., 2011, Piessens et al, 2011). *Staph. xylosus* and *Staph. sciuri*, but not *Staph. chromogenes*, showed a seasonal trends. *Staph. chromogenes* it is rarely isolated from the environment (Gillespie et al., 2009; Piessens et al., 2011). However, *Staph. sciuri* and *Staph xylosus* have been isolated from the environment of the cow: bedding or soil (Matos et al., 1991; Piessens et al., 2011). Although results of a recent molecular study have come to regard cow-to-cow transmission might play a more important role for *Staph. chromogenes* (Piessens et al., 2012), according to Taponen et al. (2008), it is a typical opportunistic skin pathogen. *Staph. sciuri* and *Staph xylosus* also have been isolated from skin (Devriese and De Keyser, 1980; Taponen et al, 2008).

In the latter group were included yeast and *Prototheca* spp. These findings have led to the conclusion that the traditional classification of these mastitis pathogens into environmental pathogens can’t be so clearly applied. As an indicator of differences in their epidemiological behavior only yeast showed a seasonal trend. As CNS included in the same group, it has been reported the existence of persistent infections by yeast and *Prototheca* spp. (Scaccabarozzi et al., 2011)

It has been demonstrated that the genetic diversity of enterococci isolated from mastitis is attributable to a probable environmental origin (Petersson-Wolve et al., 2008). There is very little information on the epidemiology of those species less frequently isolated from milk samples, such as *Enterococcus* spp. and *Lactococcus* spp. (Zadoks et al., 2011b). Since there are a few studies on these mastitis pathogens, they usually assume a similar epidemiological behavior within this group, often referred to as *Streptococcus* species other than *Strep. agalactiae*. However, our results suggest differences in the epidemiology behavior at the species level.

Results obtained in this study reflect a greater complexity in the behavior of different pathogens, unlike the traditional classification into contagious or environmental. The decrease of bulk milk somatic cell counts achieved by the mastitis control program is the result of the decrease in IMI caused by a limited number of mastitis pathogens (*Strep. agalactiae*, *Staph. epidermidis* and *Corynebacterium* spp.). Other mastitis pathogens exhibit a more or less clear trend of increasing the proportion of isolation over time and it is a reflection of the low effectiveness of traditional control measures. Time-series analysis and multivariate statistical techniques, such as Cluster Analysis, allow an adequate comparison of isolation trends of mastitis pathogens because of their ability to cope with stochastic dependence of consecutive data. Furthermore, they are a powerful tool to identify the epidemiological behavior of mastitis pathogens using the results of milk samples submitted for routine microbiological examination, by classifying them into relatively homogeneous groups.

CONFLICT OF INTEREST STATEMENT

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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Table 1. Results, the ARIMA trend model and the higher and lower monthly proportions of each mastitis pathogen isolated from milk samples. NW Spain, 2005-2011.

Caption of Figures:

Figure 1: Trend of standardized isolation proportion from total milk samples, by month and mastitis pathogen. NW Spain, 2005-2011.

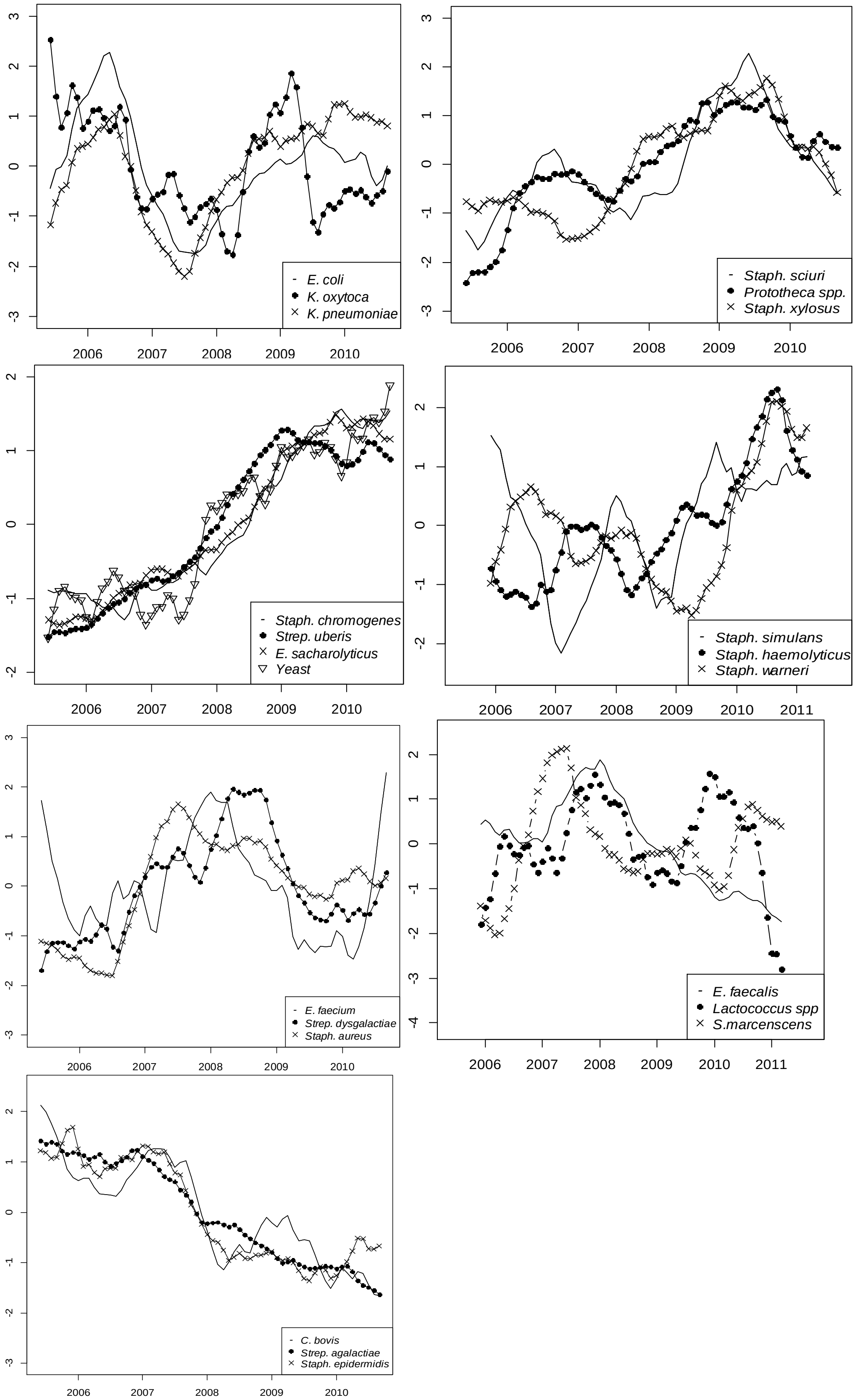
Figure 2. Dendrogram and cluster analysis of mastitis pathogens isolated from milk samples. NW Spain, 2005-2011.

Table 1. Results, the ARIMA trend model and the higher and lower monthly proportions of each mastitis pathogen isolated from milk samples. NW Spain, 2005-2011.

Pathogen	% Total (1)	Monhtly % Range(2)	Model	p-valor test Box-Ljung(3)	Monthly proportion(4)	
					Higher	Lower
Strep. uberis	12.37	7.71-17.01	ARIMA(0,1,1)(1,0,1)$_{12}$ with constant	0.5282	Juny	November
Strep. dysgalactiae	4.79	3.21-6.75	ARIMA(0,1,1) X ARIMA(0,1,1)$_{12}$ without constant	0.4921	January	August
Strep. agalactiae	2.14	0.40-4.44	ARIMA(0,1,1) with constant	0.9797		
Staph. aureus	11.28	6.64-16.20	ARIMA(0,1,1) X ARIMA (0,1,1)$_{12}$ without constant	0.772	April	August
Staph. chromogenes	1.68	0.91-2.87	ARIMA(0,1,1) with constant	0.5967		
Staph. epidermidis	1.47	0.59-3.22	ARIMA(0,1,1) with constant	0.6819		
Staph. simulans	1.16	0.49-1.88	ARIMA(0,0,0) with constant	0.1082		
Staph. haemolyticus	1.05	0.31-1.92	ARIMA(0,1,1) with constant	0.123		
Staph. xylosus	0.83	0.23-1.70	ARIMA(0,1,1) X ARIMA(0,1,1)$_{12}$ without constant	0.5771	September	March
Staph. sciuri	0.77	0.26-1.56	ARIMA(0,0,1) X ARIMA(0,0,1)$_{12}$ with constant	0.9276	October	February
Staph. warneri	0.67	0.25-1.40	ARIMA(0,1,1) without constant	0.6625		
E. coli	5.72	3.79-8.65	ARIMA(1,0,0) with constant	0.6712		
K. pneumoniae	0.96	0.44-1.88	ARIMA(1,0,0) with constant	0.1447		
S.marcescens	0.36	0.13-0.87	ARIMA(0,1,1) X ARIMA (0,1,1)$_{12}$ without constant	0.7108	January	March
K. oxytoca	0.31	0.05-0.66	ARIMA(0,0,0) with constant	0.6482		
E. faecalis	1.92	0.79-3.01	ARIMA(0,1,1) without constant	0.1315		
E. faecium	0.70	0.29-1.39	ARIMA(1,0,0) with constant	0.924		
E. sacharolyticus	0.39	0.039-1.01	ARIMA(1,1,1) with constant	0.6864		
Lactococcus spp	1.65	0.95-4.80	ARIMA(1,0,0) with constant	0.8816		
C. bovis	12.57	8.4-18.46	ARIMA(0,1,1) X ARIMA (1,1,0)$_{12}$ without constant	0.7698	September	May
Yeast	2.40	1.47-3.93	ARIMA(0,1,1) X ARIMA(0,1,1)$_{12}$ without constant	0.7421	October	April
Prototheca spp.	0.56	0.10-1.20	ARIMA(0,1,1) with constant	0. 4021		
No growth	12.72	9.9-15.6				
CS	13.67	7.5-18.9				

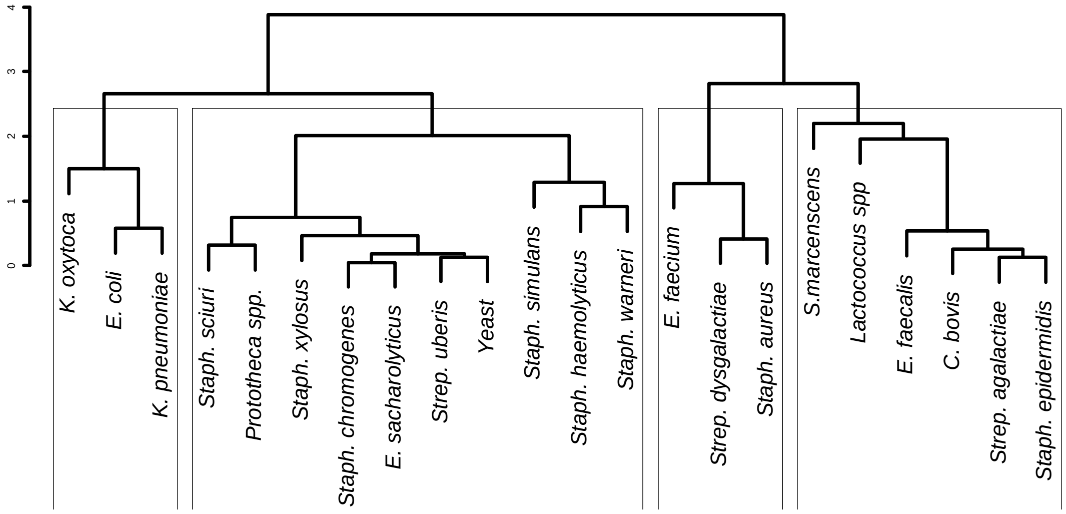
(1)Proportion of all isolations during the study
(2)Higher and lower monthly proportions
(3)Statistical test of whether any of a group of autocorrelations of a time series are different from zero.
(4)Only for mastitis pathogens which showed a seasonal trend.
CS: Contaminated samples

Figure 1: Trend of standardized isolation proportion from total milk samples, by month and mastitis pathogen. NW Spain, 2005-2011.



The Y axis represent the trend for the time series. The trend is the component of a time series that represents the behavior of the series.

Figure 2. Dendrogram and cluster analysis of mastitis pathogens isolated from milk samples. NW Spain, 2005-2011.



The dendrogram is represented by two perpendicular axes. In horizontal axis represent the elements to be sorted, on vertical axis represent the distances at which the clusters are coming joined.