Journal of
Applied Microbiology

Journal of Applied Microbiology ISSN 1364-5072

ORIGINAL ARTICLE

Relationships among hygiene indicators in take-away foodservice establishments and the impact of climatic conditions

I. Djekic[1], J. Kuzmanović[2], A. Anđelković[2], M. Saračević[2], M.M. Stojanović[2] and I. Tomašević[3]

1 Department of Food Safety and Quality Management, Faculty of Agriculture, University of Belgrade, Belgrade, Serbia
2 Center for Food Analysis, Belgrade, Serbia
3 Department of Animal Origin Products Technology, Faculty of Agriculture, University of Belgrade, Belgrade, Serbia

Keywords
climatic conditions, food safety, hygiene indicators, process hygiene, take-away establishments.

Correspondence
Ilija Djekic, Department of Food Safety and Quality Management, Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080 Belgrade, Serbia.
E-mail: idjekic@agrif.bg.ac.rs

2016/0727: received 29 January 2016, revised 23 May 2016 and accepted 17 June 2016

doi:10.1111/jam.13211

Abstract

Aims: This paper examined the relationships among hygiene indicators in take-away foodservice establishments and the impact of climatic conditions.
Methods and Results: A total of 7545 samples were collected encompassing 2050 from food handlers' (HF) hands, 3991 from stainless steel food contact surfaces (FCS) and 1504 samples from plastic FCS. The study covered a period of 43 months. Hygiene-indicator bacteria (total plate count, Enterobacteriaceae *Staphylococcus*) were determined from the samples collected from 559 different take-away establishments. Climatic conditions were evaluated in respect to the outside temperature, pressure, humidity and precipitation. Logistic regression confirmed that the presence of precipitation was associated with an increased likelihood of exhibiting both *Enterobacteriaceae* and *Staphylococcus* on HF' hands as well as exhibiting *Enterobacteriaceae* on both types of FCS. Numerable *Enterobacteriaceae* and *Staphylococcus* levels on HF' hands were detected when higher outside temperatures and higher precipitations occurred. Higher outside temperatures were observed when *Enterobacteriaceae* were detected on both plastics ($P < 0{\cdot}05$) and stainless steel ($P > 0{\cdot}05$). Higher precipitation was observed when *Enterobacteriaceae* was detected on stainless steel while in contrast, this indicator was detected on plastics in periods with lower precipitation.
Conclusions: This research confirms relationships between hygiene indicators in take-aways and climatic conditions, mostly temperature and precipitation.
Significance and Impact of the Study: This study provides another perspective into the possible nature of cross-contamination and foodborne outbreaks originating in foodservice establishments and brings to attention the necessity of analysing various climatic conditions.

Introduction

When eating outside, consumers expect safe food prepared in an acceptable hygiene environment (Djekic *et al.* 2014). Foodservice establishments are recognized as sources of foodborne outbreaks. Consumption of food contaminated with foodborne micro-organisms and toxins produced by the micro-organisms may cause death, illness and hospitalization (Yoon *et al.* 2008; Luning *et al.* 2013). Foodborne bacteria can be transmitted at different food preparation stages (Abdul-Mutalib *et al.* 2015). Foodservice establishments are considered as small scale business with a complex 'production' system, and a large numbers of inputs, processes and outputs (Taylor 2008). These establishments typically include restaurants, caterers, institutional food services, take-away places and café-bars (Djekic *et al.* 2016). The only types of foodservice establishments with food sold in an outdoor environment are 'take-aways'. These types of establishments are sometimes referred to as street food (CAC 2001) or street-vended food establishments (CAC 1997; CXP 2001). They are defined as establishments that prepare and/or sell foods and beverages by vendors in streets and other public places for immediate consumption or consumption at a later time without further processing or preparation (WHO 1996).

In controlling the spread of foodborne pathogens in foodservice establishments, special care is given to food contact surfaces (FCS) (Cosby *et al.* 2008). These food preparation and serving surfaces include food containers, slicing machines, plates, cutting boards, knives, cutlery, dishes, serving trays, serving counters and plastic recipients (Yoon *et al.* 2008; Garayoa *et al.* 2014; Lahou *et al.* 2015). Two main nonporous food contact materials common in foodservice establishments are stainless steel and plastic (D'Souza *et al.* 2006).

The risk of foodborne infection associated with cross-contamination depends on two factors: the level of contamination on the surfaces and the probability of its transfer to food (Bloomfield and Scott 1997). Foodborne diseases originate from bacterial cross-contamination of FCS and the subsequent cross-contamination to food products (Shi and Zhu 2009). Bacteria are able to survive on a variety of materials, but survival rates differ on different types of materials (Wilks *et al.* 2005). Cleaning and sanitation practices are prerequisite programs that prevent the development of biofilms and reduce the possibility of food contamination (Campdepadrós *et al.* 2012). It is commonly considered that the prevalence of pathogens is strongly associated with the 'house-flora' of the premises (Gounadaki *et al.* 2008).

The hands of food handlers (HF) are considered as vectors in the spread of foodborne disease due to poor personal hygiene and/or cross-contamination (Lues and Van Tonder 2007; Abd-Elaleem *et al.* 2014; de Oliveira *et al.* 2014; Taché and Carpentier 2014). Inadequate personal hygiene and handling of food may result in hands being contaminated with enteric pathogens (Lues and Van Tonder 2007).

To determine the microbial quality and hygiene, in the framework of verifying good hygiene/catering practices in foodservice establishments, it is a common practice to monitor the presence and levels of indicator bacteria. Organisms associated with hygiene practices include, among others, total coliforms, *Escherichia coli*, members of the family *Enterobacteriaceae* and *Staphylococcus aureus* (Lues and Van Tonder 2007). Enteric diseases may have a seasonal pattern, whereas the highest incidence of illnesses occurs during the summer period and changes in temperature and precipitation may influence transmission pathways (Liu *et al.* 2013; Holvoet *et al.* 2014). *Staphylococci* strains are present in the human nose and often contaminate hands (Lues and Van Tonder 2007).

Climatic conditions have an impact on food safety, incidence and prevalence of foodborne diseases (Miraglia *et al.* 2009; Bezirtzoglou *et al.* 2011; Lal *et al.* 2012; Holvoet *et al.* 2014). These incidences can be correlated with climate conditions under certain circumstances (Jacxsens *et al.* 2010). Temperature and precipitation patterns are closely related with not only the transport of enteric bacteria but also with their growth and survival (Liu *et al.* 2015). D'Souza *et al.* (2004) and Kovats *et al.* (2004) confirmed a positive association between monthly salmonellosis notifications and mean monthly temperature, identifying seasonal patterns (D'Souza *et al.* 2004; Kovats *et al.* 2004). In respect to analysing climatic condition and food safety, most of the published publications are related to farms, namely production of fresh fruit and vegetables (Liu *et al.* 2013; Holvoet *et al.* 2014; Kirezieva *et al.* 2015; Uyttendaele *et al.* 2015). Intensive precipitations are linked with contamination pathway of pathogens, mainly outdoors. This includes pathways from manure at livestock farms and from grazing pastures (Parker *et al.* 2010) as well as the microbial contamination of leafy vegetables through the spread of faecal waste onto the growing area, or through contaminated water (Holvoet *et al.* 2014). Analysis of the microbial profile of FCS in Spanish hotels revealed that significant differences exist in respect to different types of surfaces and seasonal variation (Doménech-Sánchez *et al.* 2011). However, there are no studies of how climatic conditions affect microbial loads and their survival in different (open-door) food establishments.

In order to improve the food safety environment and harmonize its legislation with the EU *acquis communautaire*, Republic of Serbia adopted two main documents. First is the new Food safety law (Law 2009) requiring implementation of Hazard Analysis and Critical Control Point (HACCP) principles in all types of food establishments. Second is the new food hygiene regulation (Regulation 2010) consistent with the EU Regulation 2073/2005 (EC 2005). Full implementation of both documents was set at June 01st 2011.

The objectives of this study were to examine possible correlations between the type and the levels of hygiene indicators on both FCS and HF' hands and the effects of climatic conditions, primarily temperature and precipitation in order to determine the extent of the parameters in correlation with the presence of hygiene-indicator bacteria.

Materials and methods

Sampling

Samples were taken from FCS and HF' hands collected from take-away foodservice establishments located in Belgrade (capital of Serbia). Sampling was carried out from

Table 1 Types and numbers of samples

Types of samples	Number of samples
Food handlers' hands	2050
Food contact surface - stainless steel	3991
Food contact surface – plastic	1504
Total number of take-away establishments	559

June 1st 2011 to December 31st 2014 (period of 43 months after HACCP became a mandatory requirement). All microbiological results were converted into $\log_{10}$ CFU cm^{-2}. Distribution of samples by type and number of take-away establishment is presented in Table 1. The majority of take-away establishments were visited at least once a year, depending on the type of samples—FSC and/or HF.

Microbiological methods

In respect to the new regulation, different methods were used for evaluating process hygiene (Regulation 2010). Samples were analysed for total plate count (TPC) according to ISO 4833-1:2003, *Enterobacteriaceae* consistent with ISO 21528-2:2004, *Salmonella* based on ISO 6579:2002 spp,. and coagulase positive *Staphylococcus* according to ISO 6888-1:1999. Sampling was performed using sterile cotton tipped applicators with wooden shaft individually wrapped. FCS were sampled by swabs obtained over a measured surface area using a sterile template (10 × 10 cm). The choice of FCS was based on visual observations of areas where the food products are processed and potentially exposed to contamination. Samples from HF which are in direct contact with the food were collected by rubbing a swab against the skin while the workers were on duty. After sampling, the swabs were returned in the tube with dilution liquid (Peptone salt solution) aseptically breaking the stick. The transportation of samples to a refrigerator (4 ± 2°C) was within 4 h. The samples were examined as soon as possible, but not later than 24 h after receipt at the laboratory. Sampling techniques were based on ISO 18593 (ISO 2004). All samples were analysed in an ISO/IEC 17025:2005 accredited laboratory.

Data processing and statistical methods

Samples with numbers above the limit of detection were included in the analysis. For a part of the statistical analysis TPC, *Enterobacteriaceae* and *Staphylococcus* data were transferred into three classes as follows: Class I (≤1 $\log_{10}$ CFU cm^{-2}); Class II (results between 1 $\log_{10}$ CFU cm^{-2} and 2 $\log_{10}$ CFU cm^{-2}); and Class III (results ≥ 2 $\log_{10}$ CFU cm^{-2}). The climatic parameters used in statistical analysis were mean outside temperature, mean pressure, mean humidity and accumulative precipitation calculated from the daily data of the four parameters as reported from the nearest local weather station (RHSS 2012, 2013, 2014, 2015).

Spearman rank order correlation coefficient (r_s) was calculated to measure the strength and direction of association that exists between hygiene-indicator bacteria in HF and FCS (both stainless steel and plastics), and climatic conditions. In order to take into account the samples below the limit of detection, the '$\log_{10}$ CFU cm^{-2}+1' transformation has been used. Binomial logistic regression was employed to determine the probability that the occurrence of indicator bacteria depends on the climatic parameters. Classes of hygiene indicators were expressed as percentages. Chi-Square test for association was used in analysing possible relationships between results of hygiene

Table 2 Prevalence of hygiene indicators in take-away establishments

	n (%*)	Indicator	Class I (%)	Class II (%)	Class III (%)
Food handlers' hands	2050 (100)	TPC	4 (0·2)	10 (0·5)	2036 (99·3)
	311 (15·2)	*Enterobacteriaceae*	253 (81·4)	57 (18·3)	1 (0·3)
	124 (6·0)	*Staphylococcus*	43 (34·7)	77 (62·1)	4 (3·2)
FCS - Stainless steel	3991 (100)	TPC	1413 (35·4)	2143 (53·7)	435 (10·9)
	795 (19·9)	*Enterobacteriaceae*	623 (78·4)	154 (19·4)	18 (2·3)
	19 (0·5)	*Staphylococcus*	6 (31·6)	13 (68·4)	0 (0)
FCS - Plastic	1504 (100)	TPC	26 (1·7)	1299 (86·4)	179 (11·9)
	543 (36·1)	*Enterobacteriaceae*	445 (81·9)	91 (16·8)	7 (1·3)
	20 (1·3)	*Staphylococcus*	7 (35)	13 (65)	0 (0)

TPC, Total plate count; FCS, food contact surfaces.
(*n*) represents the number of samples during the observed period; (%) represents their share in the total sample.
Legend: Class I (≤1 $\log_{10}$ CFU cm^{-2}—samples below LOD); Class II (results between 1 $\log_{10}$ CFU cm^{-2} and 2 $\log_{10}$ CFU cm^{-2}); Class III (results ≥ 2 $\log_{10}$ CFU cm^{-2}).
*Proportion of samples with TPC, *Enterobacteriaceae* or *Staphylococcus* > limit of detection (LOD); LOD for TPC ≥ 1 log CFU cm^{-2}; *Enterobacteriaceae* ≥ 1 log CFU cm^{-2}, *Staphylococcus* ≥ 1 log CFU cm^{-2}.

indicators and types of samples. Yate's correction was calculated when the expected frequency was less than 5. The level of statistical significance was set at 0·05. Statistical processing was performed using Microsoft Excel 2010 and SPSS Statistics 17.0 (IBM Corporation, NY, USA).

Results

Prevalence of hygiene-indicator bacteria on food handlers' hands and food contact surfaces

Indicator bacteria (TPC, *Enterobacteriaceae*, *Staphylococcus*) were determined from the samples collected from 559 different take-away establishments. During the observed period, a total of 2050 HF samples and 5495 FCS samples were collected (Table 2).

Chi-Square test confirmed that there was statistically significant association between the classes of TPC and type of samples ($\chi^2 = 5985{\cdot}458$; $P < 0{\cdot}05$) showing HF' hands with the highest share of results within Class III (results $\geq 2 \log_{10}$ CFU cm^{-2}) while FCS samples had the highest share of results within Class II. It is of note that stainless steel had the most samples in Class I, compared to hands and plastic surfaces. There was no statistical significance related to both *Enterobacteriaceae* and *Staphylococcus* classes and type of samples ($P > 0{\cdot}05$). The presence of positive *Salmonella* samples was identified in two FCS samples and this result was not explored further.

Presence of various hygiene-indicator bacteria on food handlers' hands

Spearman rank correlation was conducted using all non-categorical microbial data gathered during the survey. Regarding the subset of samples from HF' hand (Table 3), *Enterobacteriaceae* expressed some correlations with the other indicator organisms. TPC and *Enterobacteriaceae* as well as *Enterobacteriaceae* and *Staphylococcus* were significantly correlated with each other ($P < 0{\cdot}05$ for all) with the strongest correlations observed between TPC and *Enterobacteriaceae* ($r_s = 0{\cdot}535$) and TPC and *Staphylococcus* ($r_s = 0{\cdot}219$), while *Enterobacteriaceae* and *Staphylococcus* had a weak correlation ($r_s = 0{\cdot}089$). TPC had a weak correlation with the accumulative precipitation ($r_s < 0{\cdot}25$).

Climate change is recognized as a driving force leading to climate-induced events, which exert pressure on the current state of food safety and may have an impact on human health and society (Kirezieva *et al.* 2015). Also, an important part of any food safety system is personal hygiene (Djekic *et al.* 2011). Seasonal effects on the occurrence of hygienic indicators on HF' hands are presented in Fig. 1a,b. Presence of *Enterobacteriaceae* ranged from 9·6% (March) up to 22·5% (May). It is of note that the highest precipitation occurred in May (Fig. 1d). A logistic regression was performed to ascertain the effects of outside temperature, precipitation, TPC and *Staphylococcus* on the

Table 3 Spearman's Rho correlation coefficient between food handlers' hands and climatic conditions

Hands of food handlers	Temperature	Pressure	Humidity	Precipitation	TPC	*Enterobacteriaceae*	*Staphylococcus*
Temperature							
Coefficient		−0·267	−0·678				
N		2050	2050				
Pressure							
Coefficient	−0·267		0·048	−0·84			
N	2050		2050	2050			
Humidity							
Coefficient	−0·678	0·048		0·237			
N	2050	2050		2050			
Precipitation							
Coefficient		−0·84	0·237		0·070		
N		2050	2050		959		
TPC							
Coefficient				0·070		0·535	0·219
N				959		2050	2050
Enterobacteriaceae							
Coefficient					0·535		0·089
N					2050		2050
Staphylococcus							
Coefficient					0·219	0·089	
N					2050	2050	

TPC, Total plate count; *N*, amount of samples.
Results in tables present the combinations which showed significant correlations ($P < 0{\cdot}05$).

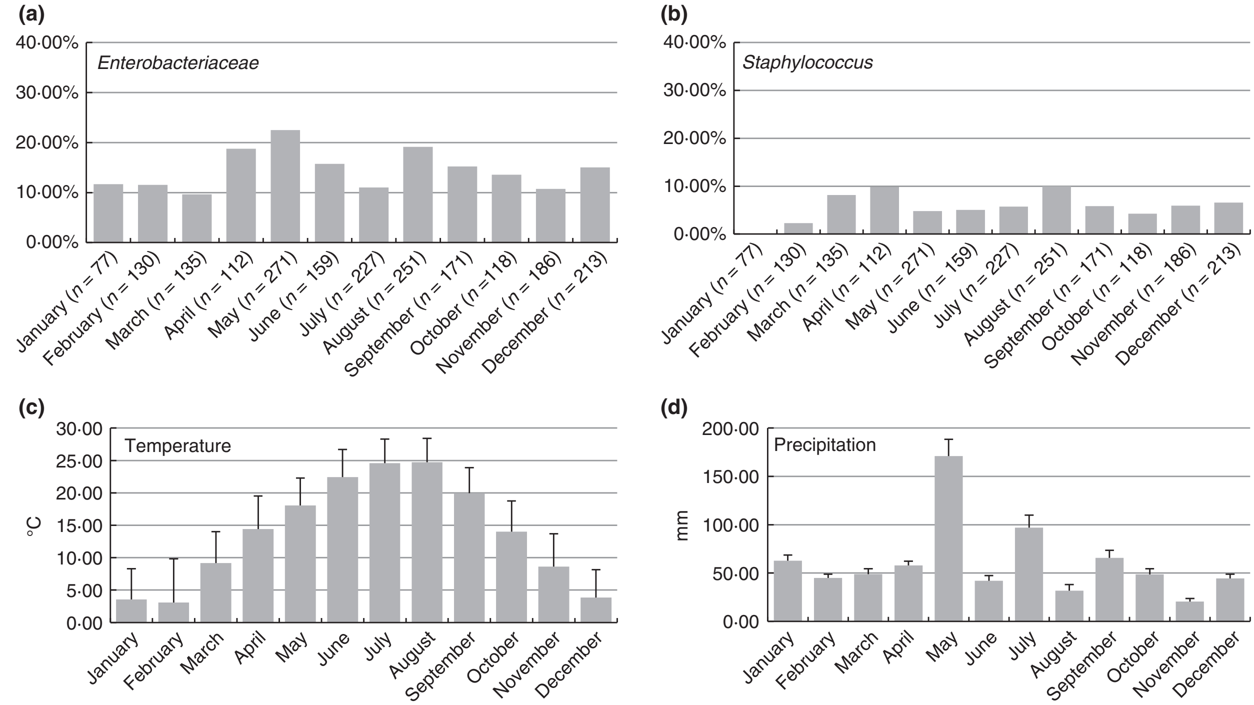

Figure 1 Seasonality of presence of indicator organisms in the various sample types in take-away establishments and characteristics of climatic conditions in function of the month throughout the sampling season. (a) *Enterobacteriaceae* presence (% > LOD) in hand swabs (n = 311); (b) *Staphylococcus* presence (% > LOD) in hand swabs (n = 124); (c) Outside temperature (43 months); and (d) Precipitation (43 months). Bars are the 95% confidence intervals and n = the amount of samples. The outside temperature and precipitation included is the mean temperature and accumulative precipitation calculated from the daily data of temperature and precipitation collected from the national hydrometeorological service.

Table 4 Spearman's Rho correlation coefficient between stainless steel food contact surfaces and climatic conditions

Stainless steel	Temperature	Pressure	Humidity	Precipitation	TPC	*Enterobacteriaceae*	*Staphylococcus*
Temperature							
Coefficient		−0·264	−0·677	0·051	0·044	0072	
N		3991	3991	1872	3991	3991	
Pressure							
Coefficient	−0·264		0·035	−0·074	−0·035		
N	3991		3991	1872	3991		
Humidity							
Coefficient	−0·677	0·035		0·249		−0045	
N	3991	3991		1872		3991	
Precipitation							
Coefficient	0·051	−0·074	0·249		0·052		
N	1872	1872	1872		1872		
TPC							
Coefficient	0·044	−0·035		0·052		0·291	
N	3991	3991		1872		3991	
Enterobacteriaceae							
Coefficient	0·072		−0·045		0·291		0·089
N	3991		3991		3991		3991
Staphylococcus							
Coefficient						0·089	
N						3991	

TPC, Total plate count; *N*, amount of samples.
Results in tables present the combinations which showed significant correlations ($P < 0·05$).

likelihood that HF were positive on *Enterobacteriaceae*. The logistic regression model was statistically significant, $\chi^2 = 456{\cdot}044$, $P < 0{\cdot}005$. The model correctly classified 86·1% of cases. Presence of precipitation and *Staphylococcus* were associated with an increased likelihood of exhibiting *Enterobacteriaceae*. TPC was a significant predictor variable in the regression model.

For the samples from HF' hands containing numerable *Enterobacteriaceae* levels, it was noted that the outside temperature (16·1°C) was significantly higher than the observed outside temperature (15·1°C) in the subset of samples showing absence of numerable *Enterobacteriaceae* (Mann–Whitney *U* test, $P < 0{\cdot}05$). Similarly, this micro-organism was detected in the period when higher precipitation occurred (6·0 mm compared to 5·8 mm; $P < 0{\cdot}05$).

Highest level of presence of *Staphylococcus* was detected in August (10·0%), April (9·8%) and March (8·1%). During the observed period, no positive samples for *Staphylococcus* were detected in January (Fig. 1b). Regression model was conducted to ascertain the effects of outside temperature, precipitation, TPC and *Enterobacteriaceae* on the likelihood that HF were positive on *Staphylococcus*. The logistic regression model was statistically significant $\chi^2 = 66{\cdot}933$, $P < 0{\cdot}005$. The model correctly classified 93·8% of cases. Presence of precipitation and *Enterobacteriaceae* were associated with an increased likelihood of exhibiting *Staphylococcus*. TPC was a significant predictor variable in the regression model.

Results with the HF samples containing numerable *Staphylococcus* levels show that the outside temperature (16·1°C) was significantly higher than the outside temperature (15·2°C) observed in the subset of samples showing absence of numerable *Staphylococcus* (Mann–Whitney *U* test, $P < 0{\cdot}05$). This micro-organism was detected in the period when higher precipitation occurred (8·3 mm compared to 5·7 mm; $P < 0{\cdot}05$). To understand the impact of climate change on food safety quantitatively, impact modelling with climate scenario analyses is needed (Jacxsens *et al.* 2010). As impact modelling require scenario analysis to generate temperature/precipitation data for food safety risk assessment (Uyttendaele *et al.* 2015), our model included these two important climatic parameters and confirmed such an influence.

Presence of various hygiene-indicator bacteria on food contact surfaces

The subset of stainless steel food contact surface samples is presented in Table 4. TPC and *Enterobacteriaceae* were significantly correlated ($r_s = 0{\cdot}291$) with each other, while *Enterobacteriaceae* and *Staphylococcus* had a weak correlation ($P < 0{\cdot}05$). TPC had low significant correlation with the mean outside temperature, mean outside pressure and the accumulative precipitation while *Enterobacteriaceae* expressed correlation with outside temperature and humidity. A recent study on correlation between food-borne bacterial pathogens and changes in air temperature and precipitation confirms various levels of correlation coefficients (Kim *et al.* 2015).

Regarding the subset of plastic contact surfaces (data not shown), all three indicator organisms were significantly correlated with each other (TPC and *Enterobacteriaceae* $r_s = 0{\cdot}538$; TPC and *Staphylococcus* $r_s = 0{\cdot}057$ and *Enterobacteriaceae* and *Staphylococcus* $r_s = 0{\cdot}101$, $P < 0{\cdot}05$). However, these indicator organisms were not significantly correlated with climatic conditions ($P > 0{\cdot}05$ for all).

Results for FCS were complemented by the binary logistic regression between indicator bacteria and climatic conditions suggesting a significant association between *Enterobacteriaceae* and climatic conditions (odds ratio >0·9). This was performed to ascertain the effects of outside temperature, precipitation and TPC on the likelihood that both types of FCS were positive on *Enterobacteriaceae*.

The logistic regression model for stainless steel was statistically significant $\chi^2 = 486{\cdot}011$, $P < 0{\cdot}005$. The model correctly classified 82·3% of cases. Occurrence of precipitation is associated with an increased likelihood of exhibiting *Enterobacteriaceae*. Results confirm that both outside temperature and TPC were significant predictor variables in the regression model.

Distribution of classes of *Enterobacteriaceae* on stainless steel FCS is presented in Fig. 2a. It shows that the majority of Class III samples ($\geq 2{\cdot}0\ \log_{10}$ CFU cm^{-2}) were detected from May to with a higher outside temperature. The temperature during sampling was the highest from 18·0°C in May, reaching its maximum of 24·7°C in August and ending in September with 19·9°C. The prevalence of *Enterobacteriaceae* in stainless steel FCS ranged from 11·8% (January) up to 27·1% (in August), Fig. 2b. Warmer temperatures alter the survival of existing pathogens and extended warmer weather seasons lengthen the period of peak incidence for many microbial diseases (Smith *et al.* 2015).

A higher (not significant) outside temperature (16·4°C) was noted when *Enterobacteriaceae* were detected on the stainless steel samples compared to 14·8°C outside temperature when this hygiene indicator was not detected. Statistically significant higher precipitation was observed when *Enterobacteriaceae* was detected (5·6 mm compared to 5·5 mm). Similar to the research of Kirezieva *et al.*, that analysed climate change driving forces (temperature, precipitation) and their pressure on the safety of fresh products (Kirezieva *et al.* 2015), our results confirm

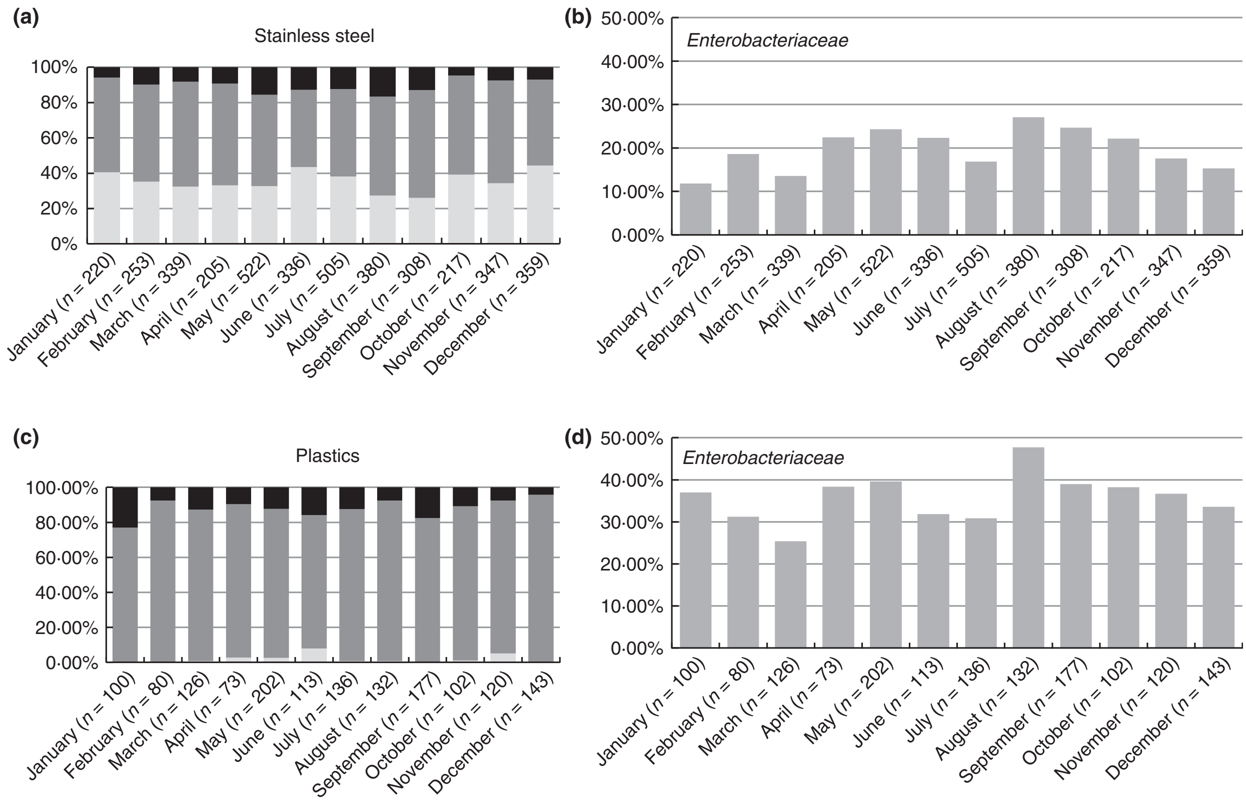

Figure 2 Seasonality of presence of indicator organisms in the various sample types in take-away establishments and characteristics of climatic conditions in function of the month throughout the sampling season. (a) Relative contributions of TPC on stainless steel food contacts surfaces (in %); Legend: Class I (≤1 $\log_{10}$ CFU cm^{-2}); Class II (results between 1 $\log_{10}$ CFU cm^{-2} and 2 $\log_{10}$ CFU cm^{-2}); Class III (≥2·0 $\log_{10}$ CFU cm^{-2}). TPC—Total plate count (n = 3991); (b) *Enterobacteriaceae* presence (% > LOD) in hand swabs (n = 795); (c) Relative contributions of TPC on plastic food contacts surfaces [in %]; Legend: Class I (≤1 $\log_{10}$ CFU cm^{-2}); Class II (results between 1 $\log_{10}$ CFU cm^{-2} and 2 $\log_{10}$ CFU cm^{-2}); Class III (≥2·0 log10 CFU cm^{-2}). TPC—Total plate count (n = 1504); (d) *Enterobacteriaceae* presence (% > LOD) in hand swabs (n = 543). Bars are the 95% confidence intervals and n = the amount of samples. (■) Class III; (■) Class II and (□) Class I.

influence of the climatic parameters on process hygiene of foodservice establishments.

The regression model for plastics was statistically significant $\chi^2 = 498{\cdot}115$, $P < 0{\cdot}005$. The model correctly classified 71·3% of cases. Occurrence of precipitation is associated with an increased likelihood of exhibiting *Enterobacteriaceae*. TPC was a significant predictor variable in the regression model. Significant association was confirmed between *Enterobacteriaceae* and climatic conditions (odds ratio >0·9, data not shown).

Distribution of classes of *Enterobacteriaceae* on plastic FCS is presented in Fig. 2c. It shows that the majority of Class III samples were detected in the period March–October. Seasonal occurrence of this hygienic indicators shows a range from 25·4% (March) up to 47·7% (in August), Fig. 2d.

As for the plastic samples containing numerable *Enterobacteriaceae* levels it was noted that the outside temperature was significantly higher (15·0°C) than the observed outside temperature in the subset of samples showing absence of numerable *Enterobacteriaceae* (14·5°C) (Mann–Whitney U test, $P < 0{\cdot}05$). In contrast, hygiene-indicator bacteria on plastics surfaces were detected in a period when lower precipitation occurred (3·9 mm compared to 4·7 mm).

Climate conditions and changes mainly impact the contamination sources and pathways of bacteria onto the primary production level during the preharvest phase where other phases of the food chain are less affected, since processing and transport are done in controlled environments (Liu *et al.* 2013). However, our research confirms that take-away foodservice establishments, being the last link in the food chain, are also affected by climatic conditions.

Discussion

This study contributes to the literature by providing another perspective into the possible nature of cross-contamination and foodborne outbreaks originating in foodservice establishments. It brings to attention the necessity of analysing various climatic conditions within ‘take-aways’ as the predominant foodservice establishment operating in an outdoor environment.

Logistic regression confirmed that the precipitation was associated with an increased likelihood of exhibiting both *Enterobacteriaceae* and *Staphylococcus* on HF’ hands. For the samples containing numerable *Enterobacteriaceae* and *Staphylococcus* levels it was noted that the outside temperature was significantly higher than the observed outside temperature in the subset of samples showing absence of numerable levels. Similar, these micro-organisms were detected in the periods when higher precipitation occurred.

Regression model confirmed that the precipitation was associated with an increased likelihood of exhibiting *Enterobacteriaceae* on both types of FCS. A higher (not significant) outside temperature was noted when *Enterobacteriaceae* were detected on stainless steel samples. Statistically significant higher precipitation was observed when *Enterobacteriaceae* was detected. Regarding plastic surfaces, it was noted that the outside temperature was significantly higher with samples containing numerable *Enterobacteriaceae* levels than the observed outside temperature in the subset of samples showing absence of numerable levels. In contrast, hygiene-indicator bacteria in plastics samples were detected when lower precipitation occurred.

Our results provide practical implications for both food microbiologists and foodservice establishment specialists. This bottom-up approach in analysing food preparation practices on-site provides added value regarding analysis of the current hygiene practices in foodservice establishments. The scientific value of this approach is the confirmation of temperature and precipitation as two climatic conditions that have an effect on the hygiene indicators in foodservice establishments operating in outdoor environments.

Several authors confirmed that survival rate of bacteria differ on different types of materials raising the question of their persistence on work surfaces (Wilks *et al.* 2005). In interpreting the results, it should be once more clarified that ‘house-flora’ (Gounadaki *et al.* 2008), type of food (Lunden *et al.* 2003), temperature, porosity and moisture of surfaces (Chevallier *et al.* 2006) and efficiency of cleaning programs (Reij and Den Aantrekker 2004) influence the hygienic status of FCS.

A limitation of this research is the fact that the authors didn’t include HF’ food safety knowledge in respect to personal hygiene and cleaning and sanitation as two prerequisite programs. Also, on-site practices within establishments, namely food handling and cleaning and sanitation practices were not analysed.

These results can be used as a basis for discussion in order to improve food preparation practices and choose alternatives to achieve better hygiene levels in establishments selling food in outdoor environments. Application of the similar method to the hygiene indicators in take-away foodservice establishments in other regions could offer a better insight into effects of climatic conditions globally.

Conflict of Interest

The authors declare that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

References

Abd-Elaleem, R., Bakr, W.M.K., Hazzah, W.A. and Nasreldin, O. (2014) Assessment of the personal hygiene and the bacteriological quality of butchers’ hands in some abattoirs in Alexandria, Egypt. *Food Control* **41**, 147–150.

Abdul-Mutalib, N.-A., Amin Nordin, S., Osman, M., Ishida, N., Tashiro, K., Sakai, K., Tashiro, Y., Maeda, T. *et al.* (2015) Pyrosequencing analysis of microbial community and food-borne bacteria on restaurant cutting boards collected in Seri Kembangan, Malaysia, and their correlation with grades of food premises. *Int J Food Microbiol* **200**, 57–65.

Bezirtzoglou, C., Dekas, K. and Charvalos, E. (2011) Climate changes, environment and infection: facts, scenarios and growing awareness from the public health community within Europe. *Anaerobe* **17**, 337–340.

Bloomfield, S.F. and Scott, E. (1997) Cross-contamination and infection in the domestic environment and the role of chemical disinfectants. *J Appl Microbiol* **83**, 1–9.

CAC (1997) *CAC/GL 22R Regional Guidelines for the Design of Control Measures for Street-Vended Foods (Africa)*. Rome, Italy: Codex Alimentarius Commission.

CAC (2001) *CAC/RCP 43R-1995, Revision 2001 - Regional Code of Hygiene Practice for Preparation and Sale of Street Foods (Latin America and the Caribbean)*. Rome, Italy: Codex Alimentarius Commission.

Campdepadrós, M., Stchigel, A.M., Romeu, M., Quilez, J. and Solà, R. (2012) Effectiveness of two sanitation procedures for decreasing the microbial contamination levels (including *Listeria monocytogenes*) on food contact and non-food contact surfaces in a dessert-processing factory. *Food Control* **23**, 26–31.

Chevallier, I., Ammor, S., Laguet, A., Labayle, S., Castanet, V., Dufour, E. and Talon, R. (2006) Microbial ecology of a small-scale facility producing traditional dry sausage. *Food Control* **17**, 446–453.

Cosby, C.M., Costello, C.A., Morris, W.C., Haughton, B., Devereaux, M.J., Harte, F. and Davidson, P.M. (2008)

Microbiological analysis of food contact surfaces in child care centers. *Appl Environ Microbiol* **74**, 6918–6922.

CXP (2001) *CXP 71R-2013 Regional Code of Practice for Street-Vended Foods (Near East)*. Rome, Italy: Codex Alimentarius Commission

Djekic, I., Tomasevic, I. and Radovanovic, R. (2011) Quality and food safety issues revealed in certified food companies in three Western Balkans countries. *Food Control* **22**, 1736–1741.

Djekic, I., Smigic, N., Kalogianni, E.P., Rocha, A., Zamioudi, L. and Pacheco, R. (2014) Food hygiene practices in different food establishments. *Food Control* **39**, 34–40.

Djekic, I., Kuzmanović, J., Anđelković, A., Saračević, M., Stojanović, M.M. and Tomašević, I. (2016) Effects of HACCP on process hygiene in different types of Serbian food establishments. *Food Control* **60**, 131–137.

Doménech-Sánchez, A., Laso, E., Pérez, M.J. and Berrocal, C.I. (2011) Microbiological levels of randomly selected food contact surfaces in hotels located in Spain during 2007–2009. *Foodborne Pathog Dis* **8**, 1025–1029.

D'Souza, R.M., Becker, N.G., Hall, G. and Moodie, K.B. (2004) Does ambient temperature affect foodborne disease? *Epidemiology* **15**, 86–92.

D'Souza, D.H., Sair, A., Williams, K., Papafragkou, E., Jean, J., Moore, C. and Jaykus, L. (2006) Persistence of caliciviruses on environmental surfaces and their transfer to food. *Int J Food Microbiol* **108**, 84–91.

EC (2005) L 338. In *Commission Regulation (EC) No 2073/2005 of 15 November 2005 on Microbiological Criteria for Foodstuffs* ed. Union, O.J.o.t.E. pp. 1–26. Brussels, Belgium: European Commision.

Garayoa, R., Díez-Leturia, M., Bes-Rastrollo, M., García-Jalón, I. and Vitas, A.I. (2014) Catering services and HACCP: temperature assessment and surface hygiene control before and after audits and a specific training session. *Food Control* **43**, 193–198.

Gounadaki, A.S., Skandamis, P.N., Drosinos, E.H. and Nychas, G.J. (2008) Microbial ecology of food contact surfaces and products of small-scale facilities producing traditional sausages. *Food Microbiol* **25**, 313–323.

Holvoet, K., Sampers, I., Seynnaeve, M. and Uyttendaele, M. (2014) Relationships among hygiene indicators and enteric pathogens in irrigation water, soil and lettuce and the impact of climatic conditions on contamination in the lettuce primary production. *Int J Food Microbiol* **171**, 21–31.

ISO (2004) *ISO 18593:2004 Microbiology of Food and Animal Feeding Stuffs - Horizontal Methods for Sampling Techniques from Surfaces Using Contact Plates and Swabs*. Geneva, Switzerland: International Organization for Standardization.

Jacxsens, L., Luning, P.A., van der Vorst, J.G.A.J., Devlieghere, F., Leemans, R. and Uyttendaele, M. (2010) Simulation modelling and risk assessment as tools to identify the impact of climate change on microbiological food safety – The case study of fresh produce supply chain. *Food Res Int* **43**, 1925–1935.

Kim, Y.S., Park, K.H., Chun, H.S., Choi, C. and Bahk, G.J. (2015) Correlations between climatic conditions and foodborne disease. *Food Res Int* **68**, 24–30.

Kirezieva, K., Jacxsens, L., van Boekel, M.A.J.S. and Luning, P.A. (2015) Towards strategies to adapt to pressures on safety of fresh produce due to climate change. *Food Res Int* **68**, 94–107.

Kovats, R.S., Edwards, S.J., Hajat, S., Armstrong, B.G., Ebi, K.L. and Menne, B. (2004) The effect of temperature on food poisoning: a time-series analysis of salmonellosis in ten European countries. *Epidemiol Infect* **132**, 443–453.

Lahou, E., Jacxsens, L., Verbunt, E. and Uyttendaele, M. (2015) Evaluation of the food safety management system in a hospital food service operation toward *Listeria monocytogenes*. *Food Control* **49**, 75–84.

Lal, A., Hales, S., French, N. and Baker, M.G. (2012) Seasonality in human zoonotic enteric diseases: a systematic review. *PLoS ONE* **7**, e31883.

Law (2009) *Food Safety Law*. Belgrade, Serbia: Official Gazette of the Republic of Serbia.

Liu, C., Hofstra, N. and Franz, E. (2013) Impacts of climate change on the microbial safety of pre-harvest leafy green vegetables as indicated by *Escherichia coli* O157 and *Salmonella* spp. *Int J Food Microbiol* **163**, 119–128.

Liu, C., Hofstra, N. and Leemans, R. (2015) Preparing suitable climate scenario data to assess impacts on local food safety. *Food Res Int* **68**, 31–40.

Lues, J.F.R. and Van Tonder, I. (2007) The occurrence of indicator bacteria on hands and aprons of food handlers in the delicatessen sections of a retail group. *Food Control* **18**, 326–332.

Lunden, J.M., Autio, T.J., Sjoberg, A.M. and Korkeala, H.J. (2003) Persistent and nonpersistent *Listeria monocytogenes* contamination in meat and poultry processing plants. *J Food Prot* **66**, 2062–2069.

Luning, P.A., Chinchilla, A.C., Jacxsens, L., Kirezieva, K. and Rovira, J. (2013) Performance of safety management systems in Spanish food service establishments in view of their context characteristics. *Food Control* **30**, 331–340.

Miraglia, M., Marvin, H.J.P., Kleter, G.A., Battilani, P., Brera, C., Coni, E., Cubadda, F., Croci, L. *et al.* (2009) Climate change and food safety: an emerging issue with special focus on Europe. *Food Chem Toxicol* **47**, 1009–1021.

de Oliveira, A.B.A., da Cunha, D.T., Stedefeldt, E., Capalonga, R., Tondo, E.C. and Cardoso, M.R.I. (2014) Hygiene and good practices in school meal services: organic matter on surfaces, microorganisms and health risks. *Food Control* **40**, 120–126.

Parker, J.K., McIntyre, D. and Noble, R.T. (2010) Characterizing fecal contamination in stormwater runoff in coastal North Carolina, USA. *Water Res* **44**, 4186–4194.

Regulation (2010) *Regulation on General and Specific Food Hygiene Requirements at any Stage of Production, Processing* *and Trade*. Belgrade, Serbia: Official Gazette of the Republic of Serbia.

Reij, M.W. and Den Aantrekker, E.D. (2004) Recontamination as a source of pathogens in processed foods. *Int J Food Microbiol* **91**, 1–11.

RHSS (2012) *Meteorological Yearbook 1 - Climatological Data for 2011*. Belgrade, Serbia: Republic Hydrometeorological Service of Serbia.

RHSS (2013) *Meteorological Yearbook 1 - Climatological Data for 2012*. Belgrade, Serbia: Republic Hydrometeorological Service of Serbia.

RHSS (2014) *Meteorological Yearbook 1 - Climatological Data for 2013*. Belgrade, Serbia: Republic Hydrometeorological Service of Serbia.

RHSS (2015) *Meteorological Yearbook 1 - Climatological Data for 2014*. Belgrade, Serbia: Republic Hydrometeorological Service of Serbia.

Shi, X. and Zhu, X. (2009) Biofilm formation and food safety in food industries. *Trends Food Sci Technol* **20**, 407–413.

Smith, B.A., Ruthman, T., Sparling, E., Auld, H., Comer, N., Young, I., Lammerding, A.M. and Fazil, A. (2015) A risk modeling framework to evaluate the impacts of climate change and adaptation on food and water safety. *Food Res Int* **68**, 78–85.

Taché, J. and Carpentier, B. (2014) Hygiene in the home kitchen: changes in behaviour and impact of key microbiological hazard control measures. *Food Control* **35**, 392–400.

Taylor, E. (2008) A new method of HACCP for the catering and food service industry. *Food Control* **19**, 126–134.

Uyttendaele, M., Liu, C. and Hofstra, N. (2015) Special issue on the impacts of climate change on food safety. *Food Res Int* **68**, 1–6.

WHO (1996) *Essential Safety Requirements for Street-Vended Foods*. Geneva, Switzerland: Food Safety Unit - Division of Food and Nutrition; World Health Organization.

Wilks, S.A., Michels, H. and Keevil, C.W. (2005) The survival of *Escherichia coli* O157 on a range of metal surfaces. *Int J Food Microbiol* **105**, 445–454.

Yoon, Y., Kim, S.R., Kang, D.H., Shim, W.B., Seo, E. and Chung, D.H. (2008) Microbial assessment in school foodservices and recommendations for food safety improvement. *J Food Sci* **73**, M304–M313.