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Editorial

Dear readers of the International Journal of Sanitary Engineering Research (IJSER), welcome to the Volume 13, Number 1, and Year 2019 of the IJSER.

Firstly, we would like to introduce you to some updates of the IJSER. We have adopted the Open Access policies in accordance with the National Strategy of Open Access to Scientific Publications and Research Data in Slovenia 2015-2020. Considering the demands of the Slovenian Research Agency, which is partially sponsoring the IJSER, we have adopted bilingual abstracts of the published articles – in English and Slovenian. The other contents of the articles are still going to be published in English only. Furthermore, we have entered into an agreement with Sciendo, which is fully owned by De Gruyter, a renowned academic publisher. Sciendo is currently publishing approximately 600 journals owned by universities and other institutions, and will raise the IJSER to the highest level.

Additionally, we would like to set out some interesting highlights of the published articles in this volume.

M. Oder and R. Fink outline biofilm on food contact materials as a public health issue in the article *Biofilm formation capacity of Bacillus cereus on silicone, polyethylene terephthalate, Teflon, and aluminium food contact materials*. Their results show what kind of the material, along with cleaning procedures and good hygiene behavior, represents the primary strategy for decreasing the risks of food poisoning in household environments. Moreover, the result indicates that biofilm biomass formation does not depend on material properties only.

The aim of the research from the United Kingdom (UK) *"How can you be allergic to peas?" – A Qualitative Study to Explore Food Handler's Knowledge, Attitudes and Understanding of Food Allergens* by D. Allen, G. Mitchell and M. Pascucilla, was to explore food handlers' knowledge, attitudes, and understanding of food allergens, according to the fact that there is an increasing proportion of the UK population who are suffering from food allergies and this combined with an increase in the frequency of eating away from home (where there is less control over the content of food) poses a significant risk.

Introducing us with the challenges and strategies in the education of primary school children related to microbiological food safety is the subject of an article by A. Ovca, M. Jevšnik, and P. Raspor. According to the findings, they consider that all the players within food supply chain and all consumers should experience proper education at the very early stage to imprint awareness about microorganisms and their role in food production, processing, distribution, preparation, and consumption.

T. Babić et al. in the article *The incidence of rotavirus infection compared to bacterial infections in different age groups of pediatric patients with gastroenteritis* investigated the presence of enteric pathogens in infants and children up to 7 years of age presenting with gastroenteritis, in the town of Niš, Serbia. Their research indicates what a substantial factor in the etiology of acute diarrheal diseases in the town of Nis is, and presents their seasonal prevalence as well.

In the end, we would like to encourage all scientists and professionals involved in the public and environmental health research to share their research findings and critical thinking of the public and/or environmental health issues with the readers of the IJSER. For more information please follow the instructions for authors published on the website of the IJSER – www.journal.institut-isi.si.

Wishing you pleasant reading.

Assist. Sara **TAJNIKAR**, MSc, BSc Sanitary Engineering
Editor

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Contents

Original scientific article

Biofilm formation capacity of *Bacillus cereus* on silicone, polyethylene terephthalate, Teflon, and aluminium food contact materials

Martina **ODER**[1], Rok **FINK**[1*]

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ABSTRACT

Biofilms on food contact materials represent public health issues because they are resistant to cleaning and disinfection. This study aims to assess the *Bacillus cereus* biofilm formation capacity on silicone, polyethylene terephthalate, Teflon, and aluminium food contact materials. The biofilm biomass was analysed with the crystal violet assay method. We used the standard strain *B. cereus* CCM 2010, wild strain *B. cereus* 100 and spores of those two strains. The results show that both the vegetative form the bacteria and it spores form large amounts of biofilm on silicone, followed by polyethylene terephthalate, Teflon, and aluminium. More detailed analysis has shown that spores form more biomass on all materials in comparison to the vegetative form and that the standard strains form low levels of biofilm in contrast to the wild strains. Selecting proper material with the lowest biofilm formation potential can prevent or reduce food contamination and consequently increase food safety.

Key words: biofilm; *Bacillus cereus*; food contact materials

POVZETEK

Biofilmi na kontaktnih površinah predstavljajo pomemben javno zdravstveni izziv, saj so bolj odporni na čiščenje in dezinfekcijo kot planktonske celice. Namen raziskave je bil ovrednotiti količino biofilma na materialih za stik z živili, kot so silikon, polietilen tereftalat, Teflon in aluminij. Količina biomase biofilma na površini je bila ocenjena z metodo kristal vijolično. V raziskavi smo uporabili standardni sev *B. cereus* CCM 2010, divji sev *B. cereus* 100 in spore obeh omenjenih sevov. Rezultati kažejo, da tako vegetativna oblika, kot spore obeh sevov tvorijo velike količine biofilma na silikonu, sledi mu polietilen tereftalat, teflon in aluminij. Bolj natančna analiza kaže, da spore tvorijo več biomase na vseh materialih v primerjavi z vegetativno obliko ter da standardni sev *B. cereus* tvori manj biofilma v primerjavi z divjim sevom. Izbira primernega materiala z najmanjšim možnim potencialom za nastanek biofilmov lahko zmanjša ali prepreči kontaminacijo živil in posledično izboljša varnost.

Ključne besede: biofilm; *Bacillus cereus*; materiali za stik z živili

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INTRODUCTION

Most household food contact materials are in permanent contact with foodstuff; therefore, the probability of acquiring surface contaminants from contact materials into the food is high [1]. The contamination of food contact surfaces during food handling due to bacteria present in foodstuff is one of the main causes of alimentary intoxication [2]. Biofilm formation is a biological phenomenon as bacteria tend to live on surfaces rather than in a planktonic state. When embedded in a biofilm, cells are protected against harsh environmental conditions, such as chemicals, physical stresses, and antimicrobial agents, because their exopolysaccharide matrices act as protective barriers that limit penetration into the biofilm [3]. Recent foodborne outbreaks have focused on biofilms on food contact materials, examining the sources of food contamination [4]. The most commonly used materials in household environments are wood, ceramics, glass, different types of metals, silicones, Teflon, and polyethylene terephthalate [5]. Those materials are used for kitchenware, such as bottles, jars, tubs, models for baking and freezing, pastry brushes, lids, pots, pans, containers, wrappings, baking sheet, milk jugs and others. *B. cereus* is a gram-positive microorganism which can form spores under harsh environmental conditions. They are pathogenic, facultative anaerobic bacteria that produce toxins. Some vegetative strains are harmful to humans and cause foodborne illness, including nausea, vomiting, and diarrhoea [6]. *B. cereus* is a pathogenic bacterium that is frequently found in various types of raw and cooked foods, and its ability to survive high cooking temperatures requires that cooked foods be served hot or cooled rapidly to prevent the growth of this bacterium [7]. Because of its ability to form highly resistant spores and its natural spread in the wild, *B. cereus* is a major food safety concern. The spores are common in soil and spread easily to cows' udders and from there to the raw milk. In addition to the ability to survive pasteurization, they also attach very well to most household materials [8] from which they can spread throughout the kitchen environment. It is well known that *B. cereus* in vegetative cells or spores tends to adhere to rough surfaces [9, 10]. One reason for this can be the presence of appendages, proteins, polysaccharides, and lipids that allow attaching and consequently forming the biofilm [11]. Moreover, some authors have reported that the surface energy of *B. cereus*, which is highly hydrophobic, is able to adhere firmly to various materials such as those found during food processing in household environments [12]. A more specific study by Ekman et al. [13] demonstrated a transfer of *B. cereus* from paper surfaces to foods. Similarly, Le Gentil et al. [14] analysed the attachment and detachment of *B. cereus* in cleaning processes and found that re-attachment can be a reason for surface contamination. Furthermore, Fink et al. [6] reported that the removal of *B. cereus* from polyurethane conveyor belts with industrial cleaning agent is difficult if not impossible. The persistence of microbial biofilms represents a significant challenge to the establishment and maintenance of hygienic conditions in different environments. The possibility of bacterial

The contamination of food contact surfaces during food handling due to bacteria present in foodstuff is one of the main causes of alimentary intoxication.

B. cereus is a pathogenic bacterium that is frequently found in various types of raw and cooked foods, and its ability to survive high cooking temperatures requires that cooked foods be served hot or cooled rapidly to prevent the growth of this bacterium.

Four different food contact materials that are often used in the home kitchen environment were tested for *B. cereus* biofilm formation: aluminium, silicon, Teflon, and poliethylenetherephalate (PET).

multiplication in foods after storage and/or handling must be taken into account when defining safe levels for human consumption [15]. The objective of this study was to analyse the capacities of *B. cereus* biofilm formation on silicone, polyethylene terephthalate, Teflon, and aluminium food contact materials and to provide consumer information on material hygiene.

METHODS

Bacteria, growth and sporulation media

In the experiment, wild strain *B. cereus* 100 (isolated from milk and kept at the University of Ljubljana, Faculty of Health Sciences), standard strain *B. Cereus* CCM 2010 (Czech Collection of Microorganisms, Brno, Czech Republic), and spores of these two strains were used.

Methods

In this study, four different food contact materials that are often used in the home kitchen environment were tested for *B. cereus* biofilm formation: aluminium, silicon, Teflon, and poliethylenetherephalate (PET). The materials were cut into the coupons of 10 × 10 mm, which were washed with 98% ethanol (Sigma-Aldrich, Misuri, ZDA) and destilled water and dried before being autoclaved. An Olympus CX40 optical microscope with an off-the-bench illuminator and CCD CMOS camera (Camera Digital microscope Electronic Eyepiece for Image) was used to visualize the structures of the materials (Figure 1). The surface roughness of the selected material was determined by mechanical profilometer Form Talysurf Series 2 from Taylor-Hobson Ltd., Leicester, Great Britain.

Determining the biofilm biomass formation capacity

Staining biofilm biomass remains a useful baseline technique to provide a practical, inexpensive, and reliable method for the detection of biofilms.

To determine the biofilm's biomass formation, a modified method by Bohinc et al. [1] and Kubota et al. [16] was used. Staining biofilm biomass remains a useful baseline technique to provide a practical, inexpensive, and reliable method for the detection of biofilms [17]. Bacteria from the collection were transferred on the nutrient agar and incubated at 37 °C 24h. After that, a single colony of strain was transferred from the nutrient agar to the nutrient broth (Biolife, Italy) and incubated under the same conditions. Next, the bacterial culture was diluted in a 1:300 ratio, with fresh nutrient broth. Sterile coupons were transferred in a sterile petri dish and exposed to the bacterial suspension; 4 mL of the nutrient broth with bacterial cultures in a ratio of 1:300 was added. The bacterial suspension and coupons were incubated for 24 hours at the temperature of 37 °C. After the incubation time, the bacterial suspension was removed and the coupons were rinsed three times with phosphate buffered saline (PBS) (80 g of NaCl, 2 g KCl, 14.4 g Na_2HPO_4, 2.4 g KH_2PO_4 in 1 L) to remove unattached or loosely attached cells. The coupons with adhered bacterial cells were exposed to 3 mL 0.1% (w/v) crystal violet suspension (Merck, Germany) for 5 min. Then the coupons were rinsed three times with the PBS

buffer to remove excess dye. In the next step, the dye was extracted from the cells with 200 μL 96% ethanol. The optical density (OD) of the ethanol/dye solution was measured with an Infinite 200® PRO microplate reader (Tecan, Austria, GmbH) at the wavelength of 620 nm (Figure 1).

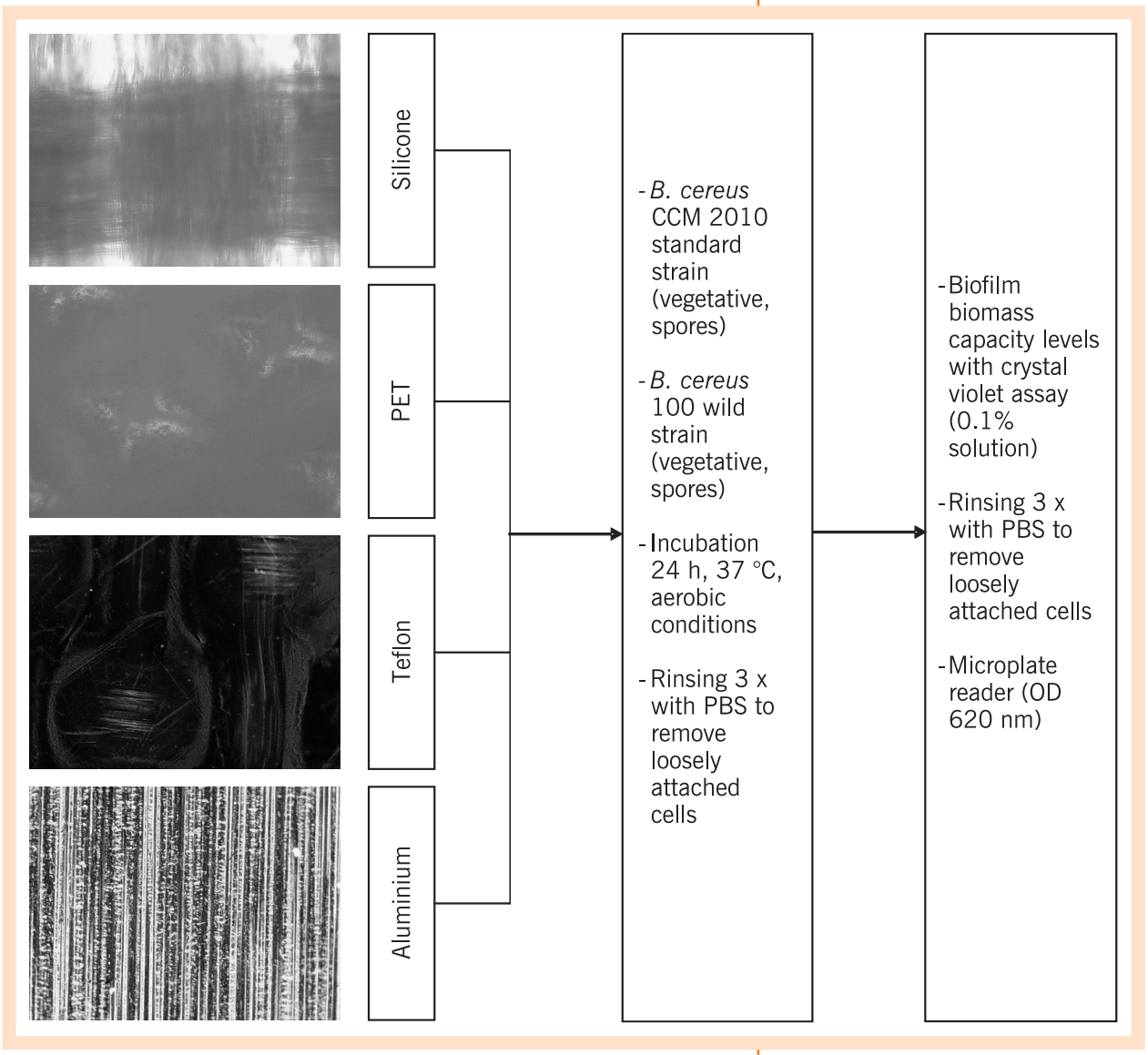

Figure 1.
B. cereus biofilm capacity assessment process flowchart.

All the experiments were performed with five parallels and three repetitions. For assay of the spores biofilm, the sporulation Casein-Casein-Yeast (CCY) medium (Sigma-Aldrich, USA) was used. The method of spore production was introduced by Abbas et al. [18] and modified as follows. To obtain spores form vegetative cells, both bacterial strains were incubated in a CCY medium for 24 hours. In the next step, bacterial culture was centrifuged with 4000 × g for 10 minutes to separate the cells from the liquid medium. The cells were re-suspended with a PBS buffer. The process was repeated three times to remove the entire liquid medium. At the final step of the culture process, the suspension was exposed to 80 °C for 10 min to destroy the

Several studies indicate that the materials of kitchen accessories (e.g. cutlery, knives, and chopping boards) represent a high risk for bacterial cross-contamination.

remaining vegetative cells. To determine the quantity of *B. Cereus* spores biofilm, the same procedure as for the vegetative form of *B. cereus* described above was used.

Statistical analysis was provided using R software version 3.1.3 and a Student's t-test comparing the OD of crystal violet dye released from the biofilm regarding the form and material. The statistical significance was set to $p < 0.05$.

RESULTS AND DISCUSSION

Food contact materials are the main source of alimentary intoxication in the domestic environment. Several studies indicate that the materials of kitchen accessories (e.g. cutlery, knives, and chopping boards) represent a high risk for bacterial cross-contamination [19]. The results of material characterization show that PET has the highest roughness of 1.2 μm, followed by silicone with 0.9 μm, Teflon 0.4 μm and aluminium with 0.2 μm. The results show that *B. Cereus* standard and wild strains, the vegetative form, and spores grow on all analysed food contact materials. The results show the least biofilm biomass on aluminium surfaces and the highest amounts on silicone (Figure 2). Furthermore, the biofilm formation capacity for standard strain *B. cereus* CCM 2010 initially inoculated from vegetative cells shows, on average, the highest biofilm capacity for silicone, followed by PET, Teflon, and aluminium (Figure 2a). Similar results can be obtained for standard strain *B. cereus* CCM 2010 inoculated from spores, for which abundant biofilm formation was found on silicone, but the fewest spores on aluminium (Figure 2b). The wild strain of *B. cereus* 100 vegetative cells formed high biofilm biomass on silicone, PET, aluminium but much less biomass was found on Teflon (Figure 2c). Complementary to that, *B. cereus* 100 wild strain biofilm inoculated from spores show the highest biofilm formation on silicone, followed by PET and aluminium. The lowest amount of biofilm inoculated from spores was found on Teflon (Figure 2d). This demonstrates that, generally (apart from PET), total amounts of biofilm biomass correspond to material roughness. It is generally accepted that the smoother the surface is, the lower the number of adhered cells is present [1, 20]. More importantly, this study indicates that a significant difference in total biofilm biomass exists when comparing material, bacterial strain, and form (vegetative form or spores).

B. cereus 100 wild strain biofilm inoculated from spores show the highest biofilm formation on silicone, followed by PET and aluminium. The lowest amount of biofilm inoculated from spores was found on Teflon.

Shaheen et al. [21] studied adhesion potential of different strains of *B. cereus* and found that spores adhere to the surface more firmly than vegetative cells do. Similar results were presented by Kolari et al. [22], who reported that hydrophobic spores of *B. cereus* are the most adhesive, one reason for which can be that strong adhesion makes favourable conditions for the spread of spores with rinse water from one location to another. Exosporium plays a significant role in spore interaction with materials, probably by providing a larger contact surface with materials. Kumariand Sarkar [23] reported that the strong adhesion potential of *B. cereus* spores has been attributed to the

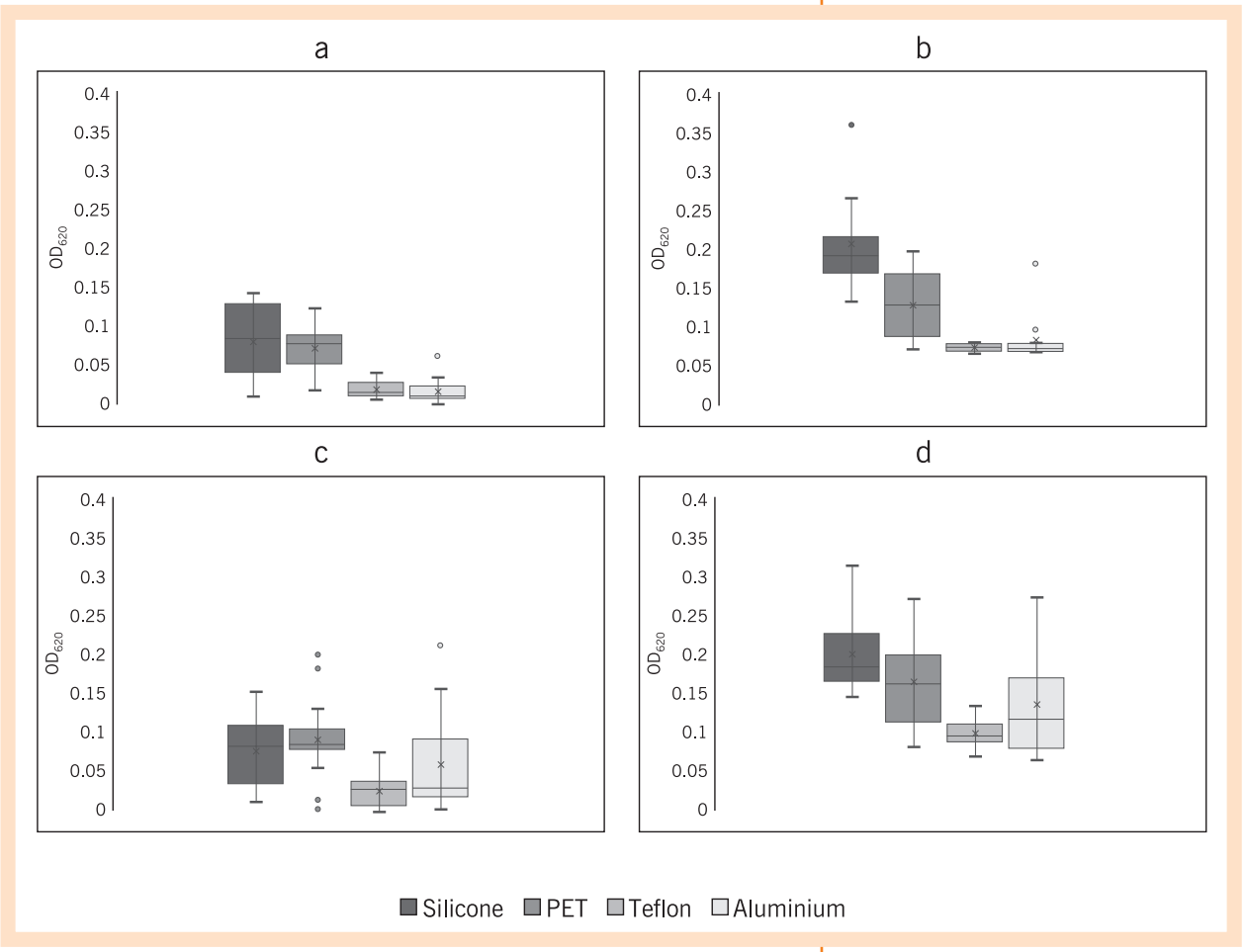

Figure 2.
Optical densities (mean, quartiles, min and max) of released crystal violet dye from *B. cereus* biofilm inoculated from vegetative form (a, b) and spores (c, d) on silicone, PET, Teflon, and aluminium.

hydrophobic character of exosporium, which varies between strains. We also found that wild strain *B. cereus* biofilm causes more biomass growth on all material in comparison to the standard strain. Comparable to our study, Hayrapetyan et al. [3] analysed standard and the undomesticated food isolate strain *B. cereus* and found significant differences in OD after 24 hours of incubation on stainless steel surfaces. Similar to that, other researchers [24, 25] reported that the amounts of biofilm biomass can vary between the strains of the same species.

Comparison of optical densities of released crystal violet dye from biofilm biomass reveals statistically significant higher optical densities for biofilm inoculated from spores on all materials and both strains ($p < 0.05$). The most abundant differences between biofilm inoculated from vegetative form and spores can be observed for silicone, in the case of both strains (ΔOD *B. cereus* CCM 2010 = 0.1317; ΔOD *B. cereus* 100 = 0.1220). In contrast, the smallest difference between biofilm inoculated from vegetative form and spores was found for Teflon when comparing the standard strain *B. cereus* CCM 2010 (ΔOD = 0.521) and the wild strain *B. cereus* 100 (ΔOD = 0.068) (Table 1).

Wild strain *B. cereus* biofilm causes more biomass growth on all material in comparison to the standard strain.

Table 1. Comparison of optical densities of crystal violet dye released from B. cereus biofilm inoculated from vegetative form and spores on silicone, PET, teflon, and aluminium.

Material	*B. cereus*	OD_{620} vegetative form	OD_{620} spores	Δ OD (/)	t-value	p-value
Silicone	Standard strain CCM 2010	0.0810	0.2127	0.1317	11.925	0.000008**
PET		0.0712	0.1438	0.0726	10.367	0.000006**
Teflon		0.0188	0.0709	0.0521	23.969	<0.000000**
Aluminium		0.0163	0.0805	0.0642	7.396	0.000049**
Silicone	Wilde strain 100	0.0778	0.1998	0.1220	9.278	0.000003**
PET		0.0921	0.1625	0.0704	8.137	0.000005**
Teflon		0.0280	0.0968	0.0688	34.32	<0.000000**
Aluminium		0.0613	0.1343	0.0730	4.085	0.001805*

Legend: * $p<0.05$; ** $p<0.000$

CONCLUSIONS

The selection of proper material with the lowest adhesion potential, along with cleaning procedures and good hygiene behaviour, represents the primary strategy for decreasing the risks of food poisoning in household environments. The results of our study demonstrated that aluminium and Teflon have much lower biofilm capacity in comparison to others. Moreover, the results of our study indicate that biofilm biomass formation depends not only on material properties but also on bacterial strain and form. By understanding the relationship between material surface properties and bacterial adhesion, strategies can be developed that would greatly inhibit, if not prevent, biofilm growth in domestic environments.

The selection of proper material with the lowest adhesion potential, along with cleaning procedures and good hygiene behaviour, represents the primary strategy for decreasing the risks of food poisoning in household environments.

Biofilm biomass formation depends not only on material properties but also on bacterial strain and form.

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Original scientific article

"How can you be allergic to peas?" – A qualitative study to explore food handler's knowledge, attitudes and understanding of food allergens

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ABSTRACT

It is clear that there is an increasing proportion of the United Kingdom (UK) population who are suffering with food allergies and this combined with an increase in the frequency of eating away from home (where there is less control over the content of food) poses a significant risk. In December 2014, the European Union (EU) introduced legislation which aimed to ensure that customers with food allergens could make informed choices and safely consume food, without the risk of a potentially life-threatening reaction. The research used semi-structured interviews with staff from a BCB, located in the North West of the UK, as the aim of the research was to explore food handlers' knowledge, attitudes and understanding of food allergens. The findings of the semi-structured interviews identified five themes: E-learning training programmes: the staff felt that these were ineffective and did not take into account individual learning styles. Responsibility: there is a lack of clarity as to who is responsible, with staff believing the key responsibility lies with the customer. Communication: similarly, communication, both within the kitchen and within the company was not clear and likely to give rise to confusion. Need to make a profit: the staff felt that the drive for profit meant that customer safety was being compromised, especially when staff numbers were reduced. Staff awareness: the staff felt confident in their own ability to prepare a safe meal but indicated that staff may be dismissive towards claims of allergen sufferers. In conclusion, these themes illustrate that a significant risk exists for allergen suffers, who rely upon the knowledge, attitudes and understanding of BCB staff to ensure their meals are safely prepared.

Key words: food allergens; food handlers; e-learning; knowledge; attitudes

POVZETEK

Jasno je, da se v Združenem kraljestvu povečuje delež ljudi, ki trpijo za alergijami na hrano, hkrati pa se povečuje pogostost prehranjevanja izven doma (kjer je manj nadzora nad vsebnostjo hrane), kar predstavlja veliko tveganje. Decembra 2014 je Evropska unija (EU) sprejela zakonodajo, ki naj bi strankam z alergijami na hrano zagotavljala dovolj informacij za izbiro in varno

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uživanje hrane, brez tveganja, da bi prišlo do smrtno nevarnih reakcij. Raziskava temelji na pol strukturiranih intervjujih z osebjem iz gostinskih podjetij na severozahodu Združenega kraljestva, saj je bil namen raziskati znanje, odnos in razumevanje alergij na hrano tistih, ki z njo rokujejo. Ugotovitve pol strukturiranih intervjujev so opredelile pet tem: Programi usposabljanja z e-učenjem: osebje meni, da so neučinkoviti in ne upoštevajo posameznih stilov učenja. Odgovornost: premalo je jasno, kdo je odgovoren, osebje pa meni, da je ključna odgovornost na strani stranke. Komunikacija: podobno tudi komunikacija, tako v kuhinji kot znotraj podjetja, ni bila jasna in bi lahko povzročila zmedo. Potreba po dobičku: osebje meni, da želja po dobičku ogroža varnost strank, še posebej ob zmanjšanju števila zaposlenih. Ozaveščenost osebja: osebje se je počutilo samozavestne glede lastne sposobnosti za pripravo varnega obroka, a se je izkazalo, da včasih ne upoštevajo zahtev ljudi, ki trpijo za alergijami. Za konec, te teme kažejo veliko tveganje za ljudi z alergijami na hrano, ki se zanašajo na znanje odnos in razumevanje osebja v gostinskih podjetjih, ki naj bi zagotavljalo, da so obroki varno pripravljeni.

Ključne besede: alergeni v hrani; rokovanje s hrano; e-učenje; znanje; odnos

INTRODUCTION

Food allergies are globally relevant and are significantly increasing in both severity and prevalence, with the data showing an upward trend to include more food product triggers. [1,2] It is estimated that between 11 and 26 million people in Europe suffer from food allergies [3], with an estimated 2 million people In the UK, living with a diagnosed food allergy [4].

It is estimated that between 11 and 26 million people in Europe suffer from food allergies.

Those suffering from food allergies can show a variety of different symptoms, ranging from mild rashes through to reactions such as anaphylactic shock, with these more serious reactions having the potential to be life-threatening. The increase in prevalence and reactions rates presents itself as a serious public health concern and one that needs to be continually monitored and addressed.

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In addition, the way people are eating is changing. Due to the increasing number of allergic people and the rise of 'eating out' culture in the UK, particularly within popular Branded Catering Businesses (BCBs) – considered to be a chain of catering establishments operating in line with a corporate personality and design – it could be argued that BCBs should now be a focal point when addressing the issue of food allergies.

Society has seen a cultural shift over recent decades with more and more people eating out and with more regularity [5]. This is reflected in the rapid growth of the hospitality sector, which is currently the third largest private sector in the UK, employing more than 3 million people and generating £130bn in economic activity. BCBs constitute a large portion of this market and are experiencing an exponential growth, resulting in thousands of people being served each day [6]. While many other businesses and companies within retail are facing uncertain futures and slipping into administration, the demand for BCBs remains.

21%-31% of allergen ingestion occurs when eating in restaurants and 13%-23% occurs in other eating out environments such as workplaces and school canteens.

According to Versluis et al., [7] 21%-31% of allergen ingestion occurs when eating in restaurants and 13%-23% occurs in other eating out environments such as workplaces and school canteens. With the large number of BCBs now operating in the UK and this level of incidents taking place outsidethe home, it is clear that practices and methods of controlling allergens within these organisations must be robust and easily understood. As there currently is no cure for food allergies, this makes avoidance the only real way for an allergic person to prevent themselves from having an allergic reaction. Therefore, the information that is presented to the consumer detailing the ingredients and content of food must be clear and accurate to allow them to make an informed choice at the point of sale [8].

As there currently is no cure for food allergies, this makes avoidance the only real way for an allergic person to prevent themselves from having an allergic reaction.

A range of legislation exists in order to protect food allergen sufferers: Article 14 of European Union (EU) Regulation 178/2002 (laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety) prohibits food from being placed onto the market if it is deemed to be either injurious to health or unfit for human consumption; Article 5 of EU regulation 852/2004 requires food retailers to have in place procedures to manage food safety based on Hazard Analysis Critical Control points (HACCP) principles and Annex II of the EU Food information for Consumers Regulation (EU) No. 11669/2011 and Commission Delegated Regulation (EU) No. 78/2014 outlines the 14 allergens that must be labelled or indicated as being present in foods (Box 1), resulting in the EU having one of the most comprehensive food allergen lists available.

The UK's response to these EU regulations, The Food Information for Consumers Regulation 2014, states that businesses must be capable of communicating to their customers which of their dishes contain any of the 14 specified allergens.

Box 1: List of EU allergens [9]

- Cereals containing gluten namely wheat (such as spelt and Khorasan wheat), rye, barley
- Crustaceans and products thereof (for example prawns, lobster, crabs and crayfish)
- Egg and products thereof
- Fish and products thereof
- Peanuts and products thereof
- Soybeans and products thereof
- Milk and products thereof (including lactose)
- Nuts (namely almond, hazelnut, walnut, cashew, pecan nut, Brazil nut, pistachio nut and Macadamia nut (Queensland nut)
- Celery and products thereof
- Mustard and products thereof
- Sesame seeds and products thereof
- Sulphur dioxide and/ or sulphites at concentrations of more than 10mg/kg or 10mg/L (litre)
- Lupin and products thereof
- Molluscs and products thereof (for example mussels, clams, oysters, scallops, snails and squid)

The preparation and handling of food in BCBs is therefore of paramount importance. Personal team members at all levels employed at BCBs need to have at least a basic knowledge of food allergies. This includes knowing the allergens and the various pathways in which an ingredient could become part of a finished dish through cross-contamination during the storage, preparation, cooking and serving stages.

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Yet staff at BCBs are often employed on minimum wage, possess few formal qualifications [10] and are young, with 29% of people employed in the industry aged under 21 years old [11]. This can lead to a high level of staff turnover, meaning that these businesses are constantly having to train new members of staff.

This should imply that BCBs are well-practiced in developing new employees to a high standard of training in a short space of time. The vast majority of BCBs have now chosen to use e-learning as their method of training staff and spend considerable sums with specialist companies in order to ensure that they are getting a product/service that is capable of delivering this. However, Sahasrabudhe and Kanungo, [12] claim the effectiveness and long-term sustainability of this training platform with regard to food service personnel's knowledge can be questioned and BCBs are now recognising that E-learning must be used in conjunction with practical training and refreshed at very regular intervals [13].

McAdams et al. [14] found that chefs were knowledgeable about food allergies and were passionate about providing safe meals to guests. However, the same study concluded that there was a general lack of access to important food allergen risk management resources and training. This is supported by Bailey et al., [15] when investigating restaurants employees' knowledge of anaphylaxis. The true or false questionnaire in this study highlighted that 90% of staff had received allergen training, yet only 50% of participants could not name more than three of these. Of the 90 participants, 80% reported that they felt confident in providing a safe meal to an allergic customer.

This shows a level of disparity and demonstrates a worrying gap in knowledge. The lack of association between the participant's knowledge and their comfort level in providing a safe meal is alarming and poses a significant danger to their customers.

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Therefore, whilst providing people with the knowledge they need to operate safely at work is essential, translating that newly acquired knowledge into behaviour change is an entirely different task, made challenging due to other variables such as attitude, beliefs and personal values [16].

Previous research has employed a quantitative approach to establishing employees knowledge and attitudes towards food allergens [17, 18, 19, 15, 20, 21, 22, 14]. From this, the researcher identified a significant lack of qualitative research into this subject. The aim of this qualitative research is to explore food handlers' knowledge, understanding and attitudes relating to food allergens and provides an opportunity to give an honest and detailed account of how food service employees perceive allergens and their feelings, thoughts and attitudes towards them.

This research design allowed the researcher to approach micro-level topics, such as the individual's views, experiences and attitudes and also explore meso-level issues around social groups, organisations and communities.

METHODS

Research design

The research took a qualitative approach and consisted of a series of semi-structured interviews with 6 team members of staff from a BCB, with each participant being asked the same questions (appendix 1). This research design allowed the researcher to approach micro-level topics, such as the individual's views, experiences and attitudes and also explore meso-level issues around social groups, organisations and communities [23]. This proved particularly beneficial when investigating how allergens are viewed across peer-groups and within a large organisation.

Sampling

This research project adopted a purposive sampling technique. Denscombe [24] believes purposive sampling provides a way of getting relevant information by selecting people most likely to have the experience or expertise to provide quality information and valuable insights in the research topic. The researcher selected participants based on their suitability and ability to contribute relevant responses to the researcher's questions. According to Malterud [25], the more information the sample holds, relevant for the actual study, the lower the number of participants is required. The figure of 6 participants was chosen as it represents one half of the whole kitchen team within the average chosen BCB (Table 1).

Table 1. Participant Information

Participant number	Length of time in industry	Current role	Training
1	16 years	Kitchen manager	Across various BCBs
2	8 years	Team leader	Across various BCBs
3	4 years	Commis chef	In- house only
4	4 years	FOH Assistant Manager to Commis chef	In- house only
5	1.5 years	Desserts	In house e-learning training
6	10 years	Sous chef	Across various BCBs Formal college qualification

Participants 1 and 2 stated they had children who were allergen sufferers and participant 3 stated they were an allergen sufferer themselves.

All participants had also undertaken the in-house, e-learning training within the last 12 months.

Semi-Structured Interviews

The questions for the semi-structure interview were generated by the author for the purpose of this research. The questions within the semi-structured interview were focused on the participants' knowledge, attitudes and understanding of food allergens.

Data collection

As this research was undertaken as part of the BSc (Hons) Environmental Health degree programme, prior to collection of any data, ethical approval was obtained from Liverpool John Moores University.

A BCB located in the North-West of England was identified and contacted asking if they would like to participate in the study. Once they had agreed, potential participants were approached in the work place and informed of the nature and purpose of the research. Confidentiality and anonymity were discussed and assured with the potential participants, as at this point concern was expressed that their comments would be fed back to their manager. Once the participants had been identified and had given consent to be interviewed, individual arrangements were made to conduct an interview with each participant. For convenience, the interviews were undertaken at participants' workplace. The interviews took place in a private function room within the workplace. The use of this room was agreed with the BCB and the interviews were made as informal as possible, with no other persons present in the room. This was important to ensure the interviewee would be in a familiar location to help them feel comfortable and eliminated any bias from management or co-workers being present. All interviews were audio recorded and upon completion of the interviews the audio recording were transcribed and all identifying information anonymised. As this was a semi-structured interview, all participants were asked the same questions as set out in the semi-structured interview and probed on answers where the interviewer wished to explore further. Participants were also able to discuss areas not covered by the semi-structured interview but related to this topic.

For convenience, the interviews were undertaken at participants' workplace. The interviews took place in a private function room within the workplace. The use of this room was agreed with the BCB and the interviews were made as informal as possible, with no other persons present in the room.

The interviewer effect was considered and throughout each interview the researcher made every effort to ensure that they maintained an unobtrusive approach, ensuring that the participant was not made to feel in any way pressured to give an untrue or inaccurate response.

Data analysis

All interviews were recorded for the purpose of transcription and each participant was allocated a number to ensure anonymity. The responses for each interviewee were transcribed and then collated for each answer. From these collated answers, the researchers examined responses and clustered similar responses together. This enabled themes to emerge and is based upon the approach identified as "cutting and sorting" by Ryan and Bernard [26]. The extraction of themes ensures validity of data in that the more frequently an experience is raised, the more authentic the theme becomes. This is to say that there may have been issues raised that were specific to an individual that did not make it into a theme as it was too unique an experience.

RESULTS

From the semi-structured interviews, the following key themes were identified:

Theme 1: Quality of in house e-learning

Theme 2: Responsibility

Theme 3: Communication

Theme 4: The impact of making a profit

Theme 5: Staff attitudes

Theme 1: Quality of in house e-learning

All the interview participants had carried out company provided e-learning within the previous year. However, when questioned, most participants struggled to explain specific allergens.

"Lupin is a type of fish, isn't it?" (Participant 1)

All participants agreed that training on allergens was important and needed to be undertaken, the method of training came under some criticism.

Whilst all participants agreed that training on allergens was important and needed to be undertaken, the method of training came under some criticism.

"Inadequate and virtually useless" (Participant 6)

"I don't count [the e-learning] as training, at the end there's a test but you can't fail it – you just redo it until you get all the answers right." (Participant 1)

The participants all held the viewpoint that the e-learning did not take into account their individual learning styles and felt that it was only provided so that the company could 'tick a box'.

The participants all held the viewpoint that the e-learning did not take into account their individual learning styles and felt that it was only provided so that the company could 'tick a box'. Participants felt that e-learning should be used in conjunction with other methods of training to help them remember of the information:

"I would prefer a classroom-based element so you can take notes back to look on, the problem with only having E-learning is that you can't go back into the training once you've done it." (Participant 4)

Participants felt that being able to engage with a person or have the opportunity to ask questions would be beneficial to them:

"E-learning was ok, but I felt very much on my own and if I didn't understand a certain thing there was no option for me to ask anybody." (Participant 6)

Participants identified the time that is required to complete the training as another problem with this training platform:

"I work long hours and only get one day off per week so having to spend that day off sat at a computer doing training is not really ideal for me." (Participant 3)

Although one interviewee working part-time had a different perspective, explaining that it is

"Convenient because I don't have to travel to work to do the training, I can just do it at home and in my own time." (Participant 5)

Theme 2: Responsibility

When asked about the responsibility around safe meal preparation, each participant gave responses that could be seen as contradictory. For instance, *Participant 3* made the following comments all in the space of one half an hour interview:

"It's the customer's responsibility to tell us that they are allergic, with the amount of people we serve everyday it just wouldn't be practical for us to ask everyone."

"It's everybody's responsibility. Someone could die if it's not done properly."

"We all know what we should do, but in practice we're too busy and the kitchen just isn't set up for it."

"It's the customer's responsibility to tell us that they are allergic, with the amount of people we serve everyday it just wouldn't be practical for us to ask everyone."

Theme 3: Communication

In terms of communication, there were two prevalent findings. The first being that communication at a central company level did not appear to be effective or monitored.

"A lot of people don't take any notice of the messages that pop up when you log on talking about a recalled batch, they just click yes and go straight to work thinking someone else will have addressed the issue." (Participant 4)

Communications between front of house staff and chefs constituted the second finding. There appeared to be some conflict between the two areas with chefs blaming front of house staff for not communicating detail around customer allergy clearly enough.

"The process is that an allergy request should be put on the ticket, then the waiter or manager comes in and tells you what it is. In reality, everyone is so busy the meal gets cooked and goes out to the customer then it bounces back because it's wrong and you have to remake the whole meal." (Participant 2)

"We get it a lot where a meal gets sent back to us from the customer who is allergic to something on the plate and we didn't get the message." (Participant 6)

Theme 4: Need to make a profit

The participants indicated that the within the company they work for, the main focus is often on how much money they can make and the focus on customer safety *"sometimes takes a back seat" (Participant 2*).

This is evident with the cutting of labour and working with a 'skeleton team' in order to increase profitability.

"Pretty much every shift now management are sending staff home halfway through a shift or only putting one or two chefs on when we really need a lot more than that to be able to do our jobs safely… This comes from head office … The management here don't have any control really, they either cut hours or get disciplinary action taken against them." (Participant 1)

The participants indicated that the within the company they work for, the main focus is often on how much money they can make and the focus on customer safety.

All participants indicated to the researcher that the speed at which food is served seemed to take priority over safety.

"If there's no lead chef of kitchen manager working on a shift because they are saving hours, then I think there's a bigger risk because it leaves us less-experienced people on shift, and we don't always know what to do." (Participant 4)

Participants did not feel that the company had the public's best interests at heart as chefs were rarely provided with the tools they need to prepare food in the right way.

"We don't get enough allergy boards and the kitchen isn't really set up for allergy prep. You just have to wipe down the section you're on before you make the meal." (Participant 5)

All participants indicated to the researcher that the speed at which food is served seemed to take priority over safety. There is a significant pressure put on food handlers to ensure the food goes out as quickly as possible. Participants explained that this pressure comes from their head office and filters down through the ranks until it reaches them.

"If we don't get food out within a certain time, we get it in the neck from our manager, so often we have to cut corners to avoid getting a bollocking." (Participant 2)

By extension, the company also appear to have a built-in way to avoid litigation through denying any and all accountability – leaving all the decision making to the customer.

"Our menu is huge, and we have so many different meals that when someone asks us if a certain allergen is in a particular dish, we just give them that book [Allergen suffering customers visiting these businesses are given information via a Food Allergen Book] to read because we don't always have the time to go and look on a box or a label." (Participant 1)

Theme 5: Staff Attitudes

Participants were asked questions about how they felt preparing and cooking a meal for an allergic guest, all six responded that they were happy to serve guests and felt very confident in their ability.

"For me it's about experience, I've been doing this for a long time now, so I know what I'm doing." (Participant 1)

There was the common consensus that customers often exaggerate or even lie about being allergic to certain foods:

"Most of the time customers just don't like a certain food but say that they're allergic.... I'm allergic to peas and mushrooms is a common one we hear; how can you be allergic to peas? You just don't want them that's all." (Participant 6).

Some participants felt strongly that older and more experienced chefs had a more relaxed and perhaps dismissive attitude towards allergens.

"I've known a few chefs who have a lot of years' experience who always say, "it was fine 10 years ago, why should things be different now?"" (Participant 3)

"Our menu is huge, and we have so many different meals that when someone asks us if a certain allergen is in a particular dish, we just give them that book [Allergen suffering customers visiting these businesses are given information via a Food Allergen Book] to read because we don't always have the time to go and look on a box or a label."

"Some more experienced chefs seem to not really give a shit that tends to be the older chefs who don't care because it never used to be talked about that much." (Participant 5).

Pressures put on chefs can also alter their attitude towards food allergens.

"Chefs don't take it as seriously here because they're under so much pressure to get the food out to the customer quickly, they get in trouble because they're falling behind which stops them taking the time they need to serve a safe meal." (Participant 4)

Limitations

The research utilised a small sample of staff within a single BCB (n=6). It must be acknowledged that for qualitative research, the participants has a degree of control over the data collected, as the researcher is unable to verify any claims made. In addition, the researcher's background was as an employee within a BCB and therefore their own experiences may influence the interpretation of the results.

DISCUSSION

Pressures put on chefs can also alter their attitude towards food allergens.

The results of the qualitative research indicated 5 key themes.

For BCBs the introduction of e-learning can be seen as a cost effective way of training staff, however, it is not without its issues. The findings, in-line with Sahasrabudhe and Kanungo (2014), indicates that its effectiveness can be questioned. The training package provided by the BCB appears to promote only surface learning (where staff are focused on only reproducing or repeating information) and not deep learning (where staff are focused on understanding information). Most staff feel it offers little value, perhaps because it does not take into account an individual's particular learning style and this suggests that there is little engagement with the training provided. A failure to fully understand what allergens are, which echoes the findings of Bailey et al. [14] and how they can contaminate food, therefore puts allergen sufferers at risk due to inappropriate practices.

E-learning can be seen as a cost effective way of training staff.

However, rather than understanding this as a type of hierarchy of responsibility, participants seemed to switch between the various levels of responsibility, thus creating a confused model of responsibility. This again leaves the allergen sufferer vulnerable, as BCB may be unwilling to take responsibility or unclear as to their own level of responsibility in relation to dealing with allergen food requests.

In terms of communication, the participants highlighted two particular areas. Firstly, the communication between the BCB and its staff. Information about the recall of potentially contaminated food is provided via the till system, yet participants admit that there is no system to verify that staff have actually read this information and participants are happy to shift the responsibility for dealing with this issue to someone else. Secondly, the communication between kitchen based staff and Front of House (FOH) based staff. The participants felt that the flow of

The participants feel that the main focus for their employer is to make money and that this can compromise allergen sufferer safety.

It is perhaps unsurprising that all participants believe that they personally would be able to provide a safe meal for an allergen suffering customer – although given the concerns around their training this could be debated.

information from the FOH staff is inadequate, the result being that meals were returned to the kitchen. The concern here is that even though the allergen sufferer may have positively engaged with the FOH to advise them of their allergen status, this message is not being transmitted to the kitchen staff. This presents situation where the allergen sufferer may consume food, thinking that based on the information they have provided, it is safe for them to eat. Interestingly, the comments focus on the inconvenience this causes for the participants and not the danger it poses to the allergen sufferer. This may be in part due to the nature of staff within the BCB, with Bolton et al. [10] and Daley [11] commenting upon the lack of formal qualification, young age and high turnover of staff typically found in BCBs.

The participants feel that the main focus for their employer is to make money and that this can compromise allergen sufferer safety; whether this be due to reducing the staff, not providing appropriate equipment and/or emphasising the need to get meals out as quickly as possible. In addition, participants felt that the BCB, in order to avoid litigation, deliberately passed the responsibility of deciding which meals would be safe to the allergen sufferer themselves (via the Food Allergen Book). This also meant that the participants did not have to spend time themselves identifying which products contained which allergens. The danger here is that should the BCB change products and fail to update its information or staff use alternative products, the allergen sufferer again is potentially exposed to a high level of risk.

It is perhaps unsurprising that all participants believe that they personally would be able to provide a safe meal for an allergen suffering customer – although given the concerns around their training this could be debated. This is consistent with the results of Ahjua and Sicher's [19] research which also identified high levels of confidence in the ability to prepare safe meals but a corresponding lack of knowledge about allergens. However, participants did indicate that other members of staff could pose a significant risk. This is based on the belief that customers are using allergens as an excuse not to eat certain types of food. These attitudes, which seem to predominate amongst the more senior members of staff, could help to influence the culture of the workplace and perceptions of more junior staff, which would perpetuate such attitudes. Regardless of how actively an allergen suffering customer engages with the staff, if they are simply not believed how can they be sure that the food produced for them is safe to eat? This perhaps reflects the idea, identified by Clayton et al. [17], that although staff are aware of the dangers, they perceive their business to below low risk.

CONCLUSION

With the rise in allergen sufferers and the popularity of BCBs, the potential for allergen- related incidents has increased. This research offers an insight into the attitudes, knowledge and understanding of BCB staff towards food allergens. Whilst the staff show some understanding of the

need to protect allergen sufferer, there appears to be a failure in accepting responsibility for this. All staff professed to be confident in their own ability to provide safe meals but less so about their willingness to accept responsibility for doing so. Staff feel that the responsibility lies with the allergen sufferer themselves. Yet the staff admit that even when the allergen sufferer engages with the process of informing the establishment, the culture and processes that exist provide multiple opportunities for this information provided to be lost or ignored. So, whilst a range of legislation may be in place to protect allergen sufferers, in order for it to be effective staff must both understand why it is there and how to comply with it – the continued failure to do so will only compromise allergen sufferer safety. Therefore, it is apparent that a cultural shift is required within BCBs, so that responsibility around food allergens does not solely lie with the allergen sufferer themselves. BCBs should ensure staff must receive appropriate and effective training on this matter, going into greater depth than the current "ticking-a-box" e-learning provision and create a culture where consumer safety is as important as profit.

Staff feel that the responsibility lies with the allergen sufferer themselves.

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Scientific review article

Challenges and strategies in the education of primary school children related to microbiological food safety – a review

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ABSTRACT

Teaching microbiological food safety and food safety in general at the primary school level is crucial, because behaviour is more easily influenced at that stage. The purpose of this review was to identify challenges in the education of primary school children related to microbiological food safety and to review the type of activities published and evaluated in the scientific literature targeting primary school children. The most frequently applied approach in the reviewed studies is target population-tailored workshops. The knowledge, attitude, and practice (KAP) model is most widely used for evaluation purposes, based mostly on the self-report and recall levels. Studies that compared theoretical and practical educational approaches unanimously demonstrated that theoretical awareness is not effective for changing and maintaining appropriate behaviour, whereas experiences had a significant impact. The review highlighted the differences and deficiencies in home economic teachers regarding formal education. The development of proper teaching methodologies and educators seems to be the key to the achievement of aware, confident, and skilled students (on the consumer level) during primary education.

Key words: food safety; education; schoolers; primary school

POVZETEK

Poučevanje temeljnih principov zagotavljanja mikrobiološke varnosti živil na ravni osnovne šole je lahko ključnega pomena, saj na tej stopnji razvoja lažje vplivamo na zavedanje in ravnanje posameznika. Namen pregleda znanstvene literature na tem področju je ugotoviti izzive, pristope in metode evalvacije pri poučevanju tovrstne tematike na ravni osnovnošolskih otrok. Najpogosteje uporabljen pristop v pregledanih študijah so usmerjene teoretične in/ali praktične delavnice, prilagojene ciljni publiki. Kot orodje za evalvacijo učinka pa se najpogosteje uporabljajo različne oblike t.i. samo poročanja udeležencev glede spremembe znanja, odnosa in ravnanja. Študije zajete v pregled dokazujejo, da imajo praktične izkušnje, ki si jih udeleženci pridobijo tekom udeležbe na delavnici pomemben pozitiven vpliv na spremembo ravnanja v povezavi z zagotavljanjem mikrobiološke varnosti živil. Razkrijejo tudi razlike in

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omejitve učiteljev gospodinjstva na področju njihove formalne izobrazbe. Razvoj ustreznih učnih metod in usposabljanje učiteljev za poučevanje mikrobiološke varnosti živil je eden ključnih korakov za doseganje osveščenih in usposobljenih bodočih potrošnikov že v času osnovnošolskega izobraževanja.

Ključne besede: varnost živil; izobraževanje; učenci; osnovna šola

Consumer behaviours, such as the ingestion of raw/undercooked foods, and poor hygiene practices are significant contributors to outbreaks of food-borne diseases.

INTRODUCTION

Foodstuffs can become a risk for consumers' health if they are not handled and treated along the food supply chain in accordance with food hygiene principles. The food supply chain does not exclude consumers, but the question is whether consumers are sufficiently informed to assure food safety at the end of the food supply chain [1, 2]. Redmond and Griffith [3] demonstrated that multiple food safety responsibilities are held by consumers, because consumers not only purchase and receive products but also process and provide foods for themselves and others. They also emphasised that the implementation of proper food-handling practices can prevent cases of food-borne disease, and the way in which consumers handle food in the kitchen affects the risk of pathogen multiplication, cross-contamination to other products, and the destruction of pathogens via thorough cooking procedures [3].

Epidemiologic surveillance summaries of food-borne diseases clearly indicate that consumer behaviours, such as the ingestion of raw/undercooked foods, and poor hygiene practices are significant contributors to outbreaks of food-borne diseases [4]. Unusan [5] reported that people of all ages seem to think they know how to handle food safely, but their self-reported food-handling behaviours do not support this confidence. Wilcock et al. [6] demonstrated that, overall, consumer attitudes towards food safety, in general, differ according to demographic and socio-economic factors, such as gender, age, educational level, and economic status. Consumers need to know which behaviours are most likely to result in illness in order to make decisions about food handling and consumption behaviours [7], and then need to be motivated to act on that knowledge as a precondition for behavioural change [8].

Consumer attitudes towards food safety, in general, differ according to demographic and socio-economic factors, such as gender, age, educational level, and economic status.

One important perspective is to educate the public about safe food handling and the preparation of foods through different kinds of educational models, which emphasise hazardous food handling techniques and the microbiological causes of food-borne disease. Education about basic food safety principles is generally emphasised as an essential factor contributing to the reduction of foodborne illnesses. As childhood usually coincides with the beginning of meal preparation experiences [9, 10, 11], it is recognised as a crucial time for developing food safety knowledge and skills [12, 13]. Once habits are established during this life period, they tend to be long-lasting and difficult to alter at later life stages regardless of the level of knowledge [14, 15]. This is

significant because children grow up and, as adults, they will continue to practice food-related behaviours at home as caregivers for family members or possibly as employees in the food business sector.

As shown by Janacsek et al. [16] the most effective time for learning new skills is from childhood to early adolescence. This is also true because children are willing to learn and have fewer previous behaviour patterns to unlearn, as already summarized by Pivarnik et al. [17]. Lavelle et al. [18] reported that learning cooking skills as a child or a teenager was positively related to the use of cooking skills, cooking practices, and cooking attitudes in later life. Food safety education can easily be incorporated into existing school curricula in courses, such as home economics or family and consumer science.

The most effective time for learning new skills is from childhood to early adolescence.

The study by Byrd-Bredbenner et al. [19] revealed that most middle schoolers are interested in food safety based on their positive attitude toward being healthy and their interest in cooking. However, as further reported by Ovca et al. [11], a high level of perceived severity and a low level of perceived vulnerability towards food-related risks are observed among this age group. In combination with confidence in their skills, this may diminish their appropriate food safety practices during food preparation, even in situations in which their knowledge is appropriate.

As further reported by others [11, 20], mothers represent the dominant sources of knowledge related to food safety (although with selective impact) and are also highly trusted sources of information. However, parental influence may be reduced in the future by teachers, peers, and celebrity chefs [21]. Nevertheless, the possibility that children educated in an effective way can act as facilitators at home through messages conveyed to family members should also be considered.

Mothers represent the dominant sources of knowledge related to food safety.

Intervention studies demonstrate a variety in teaching methods with information provision, demonstrations, and practical hands-on sessions [19]. The purpose of this review was, therefore, to identify challenges in the education of primary school children related to microbiological food safety and to review the type of activities published and evaluated in the scientific literature targeting primary school children.

METHODS

A literature review focusing on articles related to the education of primary school children related to microbiological food safety was conducted in June 2018. A search strategy was implemented in the following bibliographic databases: Scopus, Web of Sciences, and PubMed. The search algorithm comprised a targeted combination of food safety-related terms (food safety, foodhygiene), population terms (children, student) andeducational terms (education, primary school, elementary school).

The titles and abstracts of identified publications were screened for relevance to the scope of the review. Interventions in which food safety was the sole focus or part of broader nutrition-related intervention were the only studies of interest. Studies were considered for review if they

contained a food safety-related education intervention for children aged 6 to 15 years; studies that included children outside this age range were excluded. Titles and abstracts retrieved from database searches were independently screened by two authors to determine suitability for review. Relevant publications were then procured as full articles, confirmed for relevance, and reviewed in detail considering the following criteria (Type of Activity, Contents, Duration, Number of participants included, Age of participants and Evaluation procedures).

RESULTS AND DISCUSSION

Challenges

Cognitive development at this stage allows children to proceed from concrete to abstract thought, meaning that they can consider several dimensions at once and relate them in a thoughtful and relatively abstract manner [22]. However, as elaborated by different authors [12, 19], young children have difficulties comprehending *i)* microorganisms and their pathogenic effects on the human body, *ii)* why safe food handling is important, and *iii)* how to practice safe food handling. As further demonstrated by Byrd-Bredbenner et al. [19], children do not always practice safe food handling because of barriers such as hunger and the importance of other priorities despite their broad knowledge base related to safe food handling and information about how to prevent food poisoning. An additional challenge to the researchers are linguistic limitations and uncertainty about children's comprehension of the basic terms [12]. However, most children older than eleven are mature enough to participate in surveys if the language is adapted for them [23].

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If children are to be engaged in food safety education, it needs to be fun, related to real life, and provide opportunities to practice what is learned [24]. There is evidence that children find practical work relatively useful and enjoyable in comparison with other teaching activities [25]. Based on the results further reported by Byrd-Bredbenner et al. [19], children in the target group wanted food safety education to be fun, interesting, interactive, and visually intense, including hands-on learning. They also wanted the educational materials to be reflective to their lifestyles and habits. Considering the opinions of food safety experts surveyed by Byrd-Bredbenner et al. [19], food safety education should be clear, infused into existing educational activities, presented in a way that makes "it something they want to know," and marketed in a way that makes safe food-handling skills an asset.

Although schools are recognized as essential institutions influencing health-related behaviour [26], home economics as a core subject is seen as less critical than subjects where parents and other teachers perceive it as a less important subject that teaches 'lower-level' skills unlike math and science [27]. As further reported by others [28, 29], there are many barriers cited by primary school teachers to the practical model of teaching food microbiology. These include limited budgets,

poor access to resources, lack of time, lack of equipment and/or unsuitable classrooms, lack of discipline among students, lack of interest, the number of pupils in the class, and the inability to do experimental work with microorganisms. As further demonstrated by Lange et al. [30], home economics teachers' didactic choices are influenced mostly by budget, lesson time, syllabus, and their routines but also their individual experiences, knowledge and risk perception.

The challenge is linking theory and research and then further linking them to program planning andevaluation [19]. Finally, we should be aware that although teaching is essential for pupils' learning, it is no guarantee of learning [31].

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Strategies to cope with challenges

Reviewed interventions are organized in groups considering the basic approach researchers have applied (Table 1). Different types of activities with a wide range regarding the duration and number of participants are reported. However, the KAP (knowledge, attitude and practice) model is the most widely used for evaluation purposes.

Workshops

The most frequently applied approach in the reviewed studies (Table 1) is the workshop. All authors designed specifically tailored 40–90-minute workshops with the intention of promoting health and influencing the comprehension of preventive measures targeting food-related risks. The authors applied mostly cross-sectional pre-test/post-test surveys [10, 32, 33], with the exception of Traversa et al. [34] who observed participants and their replies to the questions and practical activities proposed during the workshop. For evaluation purposes, only some of them applied control group and monitored short-term and long-term effects among the target population [10, 33].

In the context of food safety, Kim and Lee [32] focused on proper hand-washing and the recognition of potentially unsafe foods. Ovca et al. [10] addressed the impact of temperature on microorganisms, the cleaning of kitchen gear, the removal of bacteria with hand washing, and the prevention of cross-contamination. Traversa et al. [34] focused on characteristics and differences among microorganisms in the context of cooking, storage, and cross-contamination. Cross-contamination was also addressed in the case of allergens. Zhou et al. [33] focused on durability (shelf life), food-related incidents, and food product safety. They were also the only one including gamification (games with an educational aspects) in the workshop through a prize contest.

Table 1. Characteristics of included studies targeting primary school-aged children with food safety-related interventions

Type of Activity	Duration	Number of participants	Age of target population	Dimensions evaluated	Reference
Workshop	40 minutes	8370	10–11	Awareness Self- reported practices	[32]
	45 minutes	671	10–12	Knowledge Susceptibility Self-reported behaviour	[10]
	90 minutes	1708	6–11	Knowledge Awareness Practice	[34]
	40 minutes	501	10–14	Knowledge Awareness Practice	[33]
Role model (Detectives, Chefs, Scientists)	6 sessions (30–60 minutes each.	561	7–9	Knowledge	[38]
	3 sessions to over a year.	86	9–11	Confidence Behaviour	[35]
	3 days	1812	8–18	Knowledge	[36]
Health promotion campaign	two lessons of two hours each	249	9–11	Knowledge Understanding Self-reported behaviour	[12, 29, 37]
Multimedia,self-paced online resource	19 days	300	11–14	Knowledge Attitude	[38]
Long-term programmes	9 months	856	10–12	Knowledge, Attitude Practice	[39]
	once a week within 50–60 minutes duration (6 months)	112	Elementary school	Knowledge Attitude Practice	[40]

As reported by Ovca et al. [10], the positive effects of workshops on knowledge and awareness are mostly of a long-term nature, showing that simplification of information and communication was on the level understood by the target group. The improvement after the intervention was much more significant if substantiated with an experiment or practical activity in comparison to the measures addressed only orally during the workshop. Furthermore, Traversa et al. [34] reported that children enthusiastically engaged with the practical experiences during the workshops, while Kim and Lee [32], who did not apply practical activity in which children would be involved, reported minor although significant improvement of self-reported hand-washing.

Role model

In this group of activities, approaches in which the target group of children was taking part in different scenarios are combined. In all three reported cases, novelty played an essential part of the delivery. Pivarnik et al. [17] developed a programme entitled *"Discovering Food Safety –*

Detective Mike Robe's Fantastic Journey" in which students and detective investigated the "mystery of food safety" to prevent a threat to food safety at a local carnival. During the programme, interactive and experiential learning techniques were applied to teach students to identify food-safety problems during food preparation and storage, to show them how to prevent potential hazards, and to recognize possible consequences of foodborne illness. During the delivery of the program, colouring books, worksheets, hands-on science experiments, a puppet show, and games designed to reinforce concepts and to evaluate knowledge gained were applied. The results and evaluations reported support the suitability of the target group. Analysis revealed a significant increase of food-safety knowledge.

The sessions with a chef were part of a national programme in the UK in which professional chefs linked with local primary schools deliver three sessions to a class covering healthy eating, practical food preparation, and a visit to a restaurant when possible [35]. The intervention had an impact on children's cooking confidence and motivated children to want to cook more. In particular, the children enjoyed having a chef delivering the session. However, a large group size and a small number of sessions left some of the children frustrated, because there was little opportunity for them to work independently.

Marklinder and Erikkson [36] introduced school-children to a scientific way of working, putting them into the role of young scientists collecting the data on refrigerator temperatures in private homes. The students were instructed to record the air temperature on three different shelves in the selected refrigerator. At each recording, the best-before date or expiry date of the existing food items was also recorded.

While Swedish teachers reported that the observational investigation increased interest and knowledge of date labelling, food hygiene, refrigerator storage, and food wastage among students [36], some US teachers preferred to have trained individuals in their classroom to execute the programme [38]. The limitation of the approach in which chefs were included is that they were without any formal nutrition or home economics training raising the issues of the correctness and consistency of the message they delivered [35].

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Health campaign

Faccio et al. [12, 37] and Losasso et al. [29] developed and evaluated a health campaign addressing the knowledge of foodborne communicable diseases, and the importance of proper food preparation and storage. They divided participants into theoretical and practical groups based on two different teaching approaches and compared pre-treatment and post-treatment data. They also investigated the potential of drawings in comparison to questionnaires and interviews as an evaluation approach.

Although both practical and theoretical approaches demonstrate improvement among the target populations, the practical one was more tailored to children's cognitive needs. The authors reported that students in the practical group represented microorganisms more in the

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context in which microorganisms are found and their actions defining through illustration of the causal linkage between the actions of microorganisms and the subsequent consequences on people. The practical group was more informed about hand-washing after touching raw meat, covering one's mouth when sneezing, and storing food in the correct way. Drawings were demonstrated to be an ecological method adapted to children's competences and abilities effective in preserving and stimulating children's creativity. The authors concluded that children were able to depict in their drawings all the critical elements corresponding to the explanations they had received in the programme [12].

Multimedia

Lynch et al. [38] developed a web-based, interactive, multimedia program engaging students in learning about food safety through the use of computers and the Internet. The web application consisted of animations, videos, games, and quizzes to convey the various food safety topics. Each lesson was delivered by an animated professor. Interactive games and activities were based on the material from the lessons, and students tested their knowledge by taking short quizzes after completing each module. The evaluation was done through pre- and post-test assessments.

Detailed analysis revealed that the web application was not appropriate for the younger grade levels (> 7th grade).

Teachers and students were excited about using the web-based program. Although the general difference between the pre-test and post-test was statistically significant,it was much smaller than expected by the authors. Detailed analysis revealed that the web application was not appropriate for the younger grade levels (> 7th grade). The authors emphasise the major advantage of this approach through the possibility of meeting the needs of all students, regardless of learning style.

Long-term programmes

Two long-term programmes are also reported in the reviewed literature (Table 1). Riyanto et el. [40] used bookcovers consisting of materials on bacteriological and chemical food safety and two 22-minute long videos. They distributed ten kinds of book covers to each student during the first week of intervention. The food safety education was given through book covers every week while videos were given three times within six months (at the beginning, in the third, and the sixth months of the intervention). The videos focused on street food practices (purchasing, reading labels) and food-borne diseases. Shen et al. [39] applied nutrition and food safety textbooks, taking into account local characteristics with several issues specifically emphasized, according to the results of the baseline investigation. During the lectures, gamification was also applied. Broadcasts and bulletins were used for educational purposes.

Both research groups applied a pre-test–post-test control group design. While Riyanto et al. [40] reported assessment done three times (before the first intervention, after the second intervention, and after the third

intervention, regarding the videos) Shen et al. [38] reported the baseline and final evaluation.

Riyanto et al. [40] reported that the knowledge, attitudes, and practices of street food safety improved significantly after six months when the pre-intervention and post-intervention results were analysed. The highest improvement was detected in the students' knowledge regarding the impact of unsafe street food on health. Shen et al. [39] reported that the programme improved knowledge and behaviour scores but had no effect on attitude.

DISCUSSION

The analysed studies (Table 1) with different intervention lengths, ranging from one school hour to a regular programme over several months with diverse outcome measurements, makes determining best practices difficult. Despite differences in delivery, each intervention had some effect on participants' knowledge, attitude and/or behaviour. Data collection methods are mostly on the self-report and recall level. However, observations [34, 35] or children's drawings [12] were also applied for evaluation purposes. As Marklinder and Erikkson [36] warned, all kinds of measurements of a self-reported nature must be interpreted with caution.

Studies that compared theoretical and practical educational approaches unanimously demonstrated that theoretical awareness is not effective for changing and maintaining appropriate behaviour, whereas experiences had significant impact [10, 12, 34, 37]. If children can participate in experiments, they see their participation as an amusing game, as further discussed by Faccio et al. [12] However, based on their literature review, Caraher et al. [35] concluded that practical cooking sessions have a greater impact on the cooking confidence of older primary school children. Furthermore, improvement of attitude is frequently evaluated, and improvement is reported [10, 38, 40]. However, as further discussed by Caraher et al. [35], a positive attitude is essential to achieving changes in behaviour, but the attitude itself does not necessarily translate into behaviour changes, whereas the latter is harder to achieve than the former. Self-efficacy, expressed as the confidence to perform food preparation activities properly, was examined only in one study [10], in which, despite their recognition that mistakes during food preparation, potentially leading to health problems, can be made, participants do not see themselves as a possible cause, also after intervention. Byrd-Bredbenner et al. [19] suggested that food safety education should equip primary schoolers with strategies to overcome barriers that are preventing the implementation of appropriate food safety practices.

If children can participate in experiments, they see their participation as an amusing game.

Furthermore, teachers' perspectives were addressed in the reviewed studies. The importance of qualified teachers, in addition to quality curriculum, was demonstrated by Pivarnik et al. [17], in which some teachers preferred to have trained individuals in their classroom to execute the intervention programme. Reservations regarding the

Policy makers have to set minimum standards regarding who is qualified to teach food safety topics on all educational levels.

pedagogical qualifications of invited chefs were also expressed by Craher et al. [35]. While Caraher [41] reported a lack of trained home economists in the UK, Ovca et al. [42] reported differences and deficiencies in Slovenian home economic teachers' formal education. Therefore, teacher education must not be neglected. One purpose of teacher education must be concerned with the issue of helping them to develop their understanding of the scientific concepts on which their teaching is based so that they feel skilled in teaching the concepts to children. Additionally, policy makers have to set minimum standards regarding who is qualified to teach food safety topics on all educational levels.

Most of the reviewed studies are short-term interventions in 40–90-minute workshops, up too few sessions in a certain period. Only two long-term programmes are described and evaluated in the reviewed literature [39, 40]. Future school-based initiatives should take the time component into account as previous research has found an association between educational exposure and desired behaviour. Despite reports that food safety contents have been restricted in national curriculums or moved from compulsory to elective courses [43, 44], as a course, home economics represents the most suitable vehicle to systematically address challenges and to implement strategies in the education of primary school children related to microbiological food safety. Home economics education (among other courses) emphasizes critical thinking and holistic approaches [27]. From their qualitative findings, Brennan et al. [45] suggested that (besides gender and work status) formal home economics training plays a determining role in domestic food safety behaviour among adult consumers. Byrd-Bredbenner et al. [19] recommend a student-centred approach involving youth in the development and design of the education about food safety. With this approach, food safety education would have characteristics desired by youth. The importance of considering children's views and perceptions is also emphasised by Lange [46].

Given the considerable number of food-borne diseases occurring in domestic food preparation, it is obvious that we do not have GHKP, and we neglect the fact that the consumer is a crucial link in the food supply chain.

It has been shown that the present maintenance of food safety in the food supply chain can easily break down, because of different kind of barriers or simple misunderstandings [47, 48]. In the classic food supply chain strategy, all relevant activities are taken for the benefit of human beings, but the consumer is located outside the system. The consumer should be an integral part of food safety systems, because he/she is a vital link between retail and home. We expected that a well-informed consumer would start to follow 'Good Housekeeping Practice' (GHKP), which is a selection of the principles and techniques of food storage and preparation at home performed directly by the consumer [47]. Given the considerable number of food-borne diseases occurring in domestic food preparation, it is obvious that we do not have GHKP, and we neglect the fact that the consumer is a crucial link in the food supply chain. Consumer behaviour and attitudes toward food safety show that the levels of understanding, motivation and trust need to be further cultivated.

CONCLUSIONS

Teaching microbiological food safety and building awareness about food microbiology at the primary level is crucial, because behaviour is more easily influenced at that stage. Learning about food safety in schools makes it possible to influence children's behaviour with systemic measures; school-based education (on the primary level) in developed countries generally reaches all social classes. The development of proper teaching methodologies seems to be the key for the achievement of aware, confident and skilled students (on the consumer level) during primary education.

Raspor and Jevšnik [2] emphasised that it should be considered that safe food is the aim of all; therefore, every misleading act and information (intentional or unintentional) that could happen in the food supply chain, in the end, affect consumers. However, the status of food safety is the result of several factors, not only effective education, starting with a favourable domestic environment. All the players within food supply chains and all consumers have to experience proper education at the very early stage to imprint awareness about microorganisms and their role in food production, processing, distribution, preparation, and consumption. This is why we shall permanently improve and adapt teaching techniques to cope with the state of the art in fast changing societies around the globe.

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Original scientific article

The incidence of rotavirus infection compared to bacterial infections in different age groups of pediatric patients with gastroenteritis

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ABSTRACT

Rotavirus is the important cause of acute gastroenteritis in pediatric patients. The aim of the present research was to determine the incidence of rotavirus infections in infants and children up to seven years of age in the town of Niš. Seasonal prevalence of rotavirus-associated acute gastroenteritis was also evaluated. An enzyme immunoassay (RIDASCREEN® Rotavirus; R-Biopharm AG, Darmstadt, Germany) was used to detect rotavirus in the stool specimens of 1,156 patients (newborns up to 7 years of age) presenting with gastroenteritis. Identification of bacteria and yeasts was performed by classical methods. The overall incidence of rotavirus in examined children was 5.97%. Among 144 hospitalized children, rotavirus infection was diagnosed in 28 (19.44%). In 1,012 children treated in outpatient setting for diarrheal diseases, rotaviruses were found in 41 (4.05%). The highest incidence of rotavirus infection was among the patients of one year of age. Among 1,156 pediatric children tested, bacterial pathogens were found in 6.31% and the most frequently isolated pathogens were *Campylobacter* spp. and *Salmonella enteritidis.* The highest prevalence of GE was recorded in the colder season, peaking in April (15.94%). Rotaviruses are an important factor in the etiology of the acute diarrheal diseases, especially in children hospitalized during the winter/spring season.

Key words: rotavirus; gastroenteritis; incidence; children

POVZETEK

Rotavirus je pomemben vzrok akutnega gastroenteritisa pri otrocih. Namen predstavljene raziskave je ugotoviti pojavnost rotavirusnih okužb pri dojenčkih in otrocih do sedmega leta starosti v mestu Niš. Ocenjena je bila tudi sezonska razširjenost rotavirusa povezanega z akutnim gastroenteritisom. Za odkrivanje rotavirusa v vzorcih blata pri 1156-ih bolnikih (novorojenčki in otroci do sedmega

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leta starosti) z gastroenteritisom, je bil uporabljen encimski imuno test (RIDA-SCREEN® Rotavirus; R-Biopharm AG, Darmstadt, Germany). Identifikacija bakterij in kvasovk je bila izvedena s klasičnimi metodami. Skupna pojavnost rotavirusa med pregledanimi otroci je bila 5,97 %. Med 144 hospitaliziranimi otroki, je okužba z rotavirusom bila diagnosticirana pri 28-ih (19,44 %). Pri 1012 otrocih, ki so jih zdravili v ambulanti, zaradi diarejskih bolezni, so rotavirusi bili najdeni pri 41-ih (4,05 %). Najvišja pojavnost rotavirusnih okužb je bila pri enoletnih otrocih. Med 1156 testiranimi otroci, so bili bakterijski patogeni najdeni pri 6,31 %, najpogosteje izolirani patogeni so bili *Campylobacter* spp. in *Salmonella enteritidis.* Največja razširjenost GE je bila zabeležena v hladnejši sezon, z vrhuncem v aprilu (15,94 %). Rotavirusi so pomemben dejavnik v etiologiji akutnih diarejskih bolezni, še posebej pri otrocih hospitaliziranih v zimski/pomladni sezoni.

Ključne besede: rotavirus; gastroenteritis; pojavnost; otroci

INTRODUCTION

Rotavirus (RV) has been shown to be the leading cause of severe acute diarrhea in pediatric patients.

Rotavirus (RV) has been shown to be the leading cause of severe acute diarrhea in pediatric patients [1, 2]. This segmented double-stranded RNA (60-80nm) viruses belong to the family Reoviridae. They are classified into seven serogroups (A-G) based on electrophoretic mobility of genomic segments and group-specific VP6 antigen of the internal capsid [3]. Further division to serotypes is based on dominant antigens VP4/VP7 of external capsid. Viruses of the serogroup A cause human disease and serogroups B and C are rarely involved [4].

Seasonal distribution of rotavirus cases may vary by geographical location.

Clinical presentation of human RV infection can vary from asymptomatic, mild or severe gastroenteritis (GE) disease that can result in a lethal dehydration. Clinical manifestations are a sudden onset of vomiting, diarrhea lasting 5–6 days on the average, and dehydration [5]. In some patients, mucosal infection can spread systemically with viral replication in different parts of the body [6]. Oral rehydration corrects the electrolyte and water loss, indicating that enterocytes in the small intestine have a functional sodium-glucose co-transporter [7].

The highest rate of infection has been reported in patients 6 to 24 months of age [8] in both developed and developing countries. However, deaths due to RVGE (Rota viral gastroenteritis) occur mainly in the low income countries of Asia, Africa and Latin America among the children below 5 years of age [9]. RV causes almost 111 million gastroenteritis (GE) episodes, 25 million clinical visits, 2 million hospitalizations and more than 453,000 deaths each year [10].

Seasonal distribution of rotavirus cases may vary by geographical location: in temperate climates most cases are observed in fall, winter and spring months [11], while in tropical countries there are not seasonal peaks [12].

Electron microscopy analysis has proven to be a valuable technique in the diagnosis of these agents in patients with acute infections. However, enzyme immunoassays are rapid, sensitive, and specific alternatives to electron microscopy providing the detection of infectious agents in

diarrheal stools specimens [13]. There are several commercial enzyme immunoassay kits which enable detection of all serotypes of group A rotaviruses [14]. Molecular techniques, for example reverse transcription-polymerase chain reaction (RT-PCR), enable diagnosis and identification of all serogroups and serotypes of human rotavirus [15].

Limited data exist on the importance of rotavirus in the etiology of diarrheal disease in all parts of Serbia due to a lack of detection and reporting policy of microbiological laboratories.

The objectives of the present prospective study was to determine the incidence of rotavirus infection in infants and children up to seven years of age presenting with GE, distribution of infection by the factors of age and gender, association between the infection caused by rotaviruses and bacteria, and seasonal prevalence of rotavirus-associated acute gastroenteritis.

Limited data exist on the importance of rotavirus in the etiology of diarrheal disease in all parts of Serbia due to a lack of detection and reporting policy of microbiological laboratories.

METHODS

Patients

In the Center for Microbiology, Public Health Institute Niš, stool specimens from 1,156 pediatric patients (newborns up to 7 years of age) presenting with GE were processed for bacteriology testing and for detection of rotaviruses over a two-year period (January 2009 – July 2010).

Rotavirus detection

Rotavirus was detected from fresh, unfrozen samples of diarrheal stools. An enzyme immunoassay (RIDASCREEN® Rotavirus; R-Biopharm AG, Darmstadt, Germany) was used to detect rotavirus in the stools specimens according to the manufacturer's instructions.

Bacterial detection and identification

For bacterial detection, fecal samples were processed by standard bacteriological methods for *Salmonella* spp., *Shigella* spp., *Yersinia enterocolitica (Y. enterocolitica)*, *Bacillus cereus* (*B. cereus*), and *Campylobacter* spp. Diarrheal toxins of *B. cereus* were confirmed using the GLISA-Rapid Test (Gold Labelled ImmunoSorbent Assay) for the qualitative detection of enterotoxin producing *B. cereus*: Duopath® Cereus for the determination of the NHE and HBL enterotoxins (Merck KGaA Darmstadt, Germany). Yeasts were detected to the genus level on blood agar.

Statitistical methods

Data were analyzed using the Excel (Microsoft) and SPSS software (SPSS). In order to determine the differences in the proportions of RVGE cases for the factors of gender, age, hospitalization and seasonal of distribution of RV, the two-tailed P value χ^2 test was used. The result was significant at $p < 0.05$.

RESULTS

A total of 1,156 children below eight years of age with acute GE were included in the study, with a mean age of 2.2 years. There were 144 hospitalized and 1,012 out-patients (Table 1). Pathogens were detected in 139 (11.42%) stool samples taken from 1,156 subjects. For all tested pathogens, 1,024 (88.58%) samples were negative. RVGE and bacterial gastroenteritis were detected in 69 and 70 cases, respectively. There was not a significant difference between the frequency of RV and bacterial infection ($p = 0.794$).

Table 1. Detection of rotaviruses in stools samples of infants and younger children regarding gender and hospitalization

Investigated groups	Number of subjects (%)	Sex (%)		Number of positive (%)	Sex (%)	
		Male	Female		Male	Female
Hospitalized patients	144 (12.46)	87 (60.42)	57 (39.58)	28 (19.44)	17 (60.71)	11 (39.29)
Outpatients	1012 (87.54)	577 (57.02)	435 (42.98)	41 (4.05)	24 (58.54)	17 (41.46)
Overall	1156 (100)	664	492	69	41 (59.42%)	28 (40.58%)

In hospitalized children with severe disease symptoms, rotaviral infection was diagnosed in 19.44% patients.

The overall incidence of rotavirus as the most frequent pathogen in the examined 1,156 children presented with RVGE (newborns up to 7 years of age) was 5.96% (69/1,156). Bacterial enteric pathogens of acute gastroenteritis were *Salmonella* spp., *Campylobacter* spp., *Yersinia enterocolitica* O3, *Shigella flexneri*, *Bacillus cereus*, with detection rate of 6.23% (72/1156). In one patient, *Candida* spp. was detected.

In hospitalized children with severe disease symptoms, rotaviral infection was diagnosed in 19.44% (28/144) patients. Out of 87.54% (1012/1156) ambulatory treated pediatric patients, 4.05% (41/1012) had rotavirus in their stool samples (Table 2). A significant difference was found in the frequency of rotavirus detection between hospitalized children and those who were in community ($\chi^2 = 53.2197$; $p < 0.001$).

The overall highest incidence of RV infection (35.00%) was among the hospitalized infants of one year of age (Table 2). The incidence of rotavirus infection in outpatients was in the range of 1.43% to 10% (Table 2).

Age distribution of acute gastroenteritis cases is presented in Table 4. The cumulative age distribution for RV-positive cases was 75.4% (n = 52) for patients younger than 24 months ($P < 0.05$) and 95.7% (n = 66) in those younger than five years. When the dataset was analyzed according to the age groups, for the first 24 months (n = 52, 59.8%), rotavirus was the main pathogen ($p < 0.05$), whereas for ages 25 – 60 months (n = 20; 47.6%), it was *Salmonella* spp. ($p < 0.05$) (Table 5). Furthermore, *Campylobacter* spp. was the main pathogen for children older than five years, but this difference was not significant ($p = 0.629$).

In the 0-24 months age group (n = 87; 63.04%), the cases of RV (n = 52; 59.77%) outnumbered the bacterial agents (n = 23; 26.44%). In the two-five years age group (n = 42; 30.43%), the main bacterial agent was *Salmonella* spp. (68.96%). In nine patients older than five

Table 2. The presence of *RVGE in different age groups

Age	Hospitalized patients		Outpatients		Total patients	
	No	%	No	%	No	%
0 – 6 months	3/31	9.68	3/87	3.45	6/118	5.08
6 months – 1 year	9/41	21.95	11/205	5.37	20/246	8.13
1 year	7/20	35.00	7/183	3.83	14/203	6.90
2 years	4/19	21.05	8/154	5.19	12/173	6.94
3 years	1/9	11.11	4/117	3.42	5/126	3.97
4 years	3/12	25.00	3/92	3.26	6/104	5.76
5 years	0/6	0	3/94	3.19	3/100	3.00
6 years	0/3	0	1/70	1.43	1/73	1.37
7 years	1/3	33.33	1/10	10	2/13	15.38
Total	28/144	19.44	41/1012	4.35	69/1156	5.96

*RVGE – Rotaviral gastroenteritis

Table 3. Detection of patients positive only to *RVGE in total number of patients

Age	Positive hospitalized patients		Positive outpatients		Total number of positive patients	
	No	%	No	%	No	%
0 – 6 months	3	10.71	3	7.32	6	8.69
6 months – 1 year	9	32.15	11	26.82	20	28.98
1 year	7	25.00	7	17.07	14	20.29
2 years	4	14.29	8	19.51	12	17.39
3 years	1	3.57	4	9.76	5	7.25
4 years	3	10.71	3	7.32	6	8.70
5 years	0	0.00	3	7.32	3	4.35
6 years	0	0.00	1	2.44	1	1.45
7 years	1	3.57	1	2.44	2	2.90
Total	28	100	41	100	69	100

*RVGE – Rotaviral gastroenteritis

Table 4. Total pathogens distribution according to the patients' age

Age groups, ‡mo	No.	The number of identified pathogens, N° (%)						
		†RV	*Salmonella*	*Campylo-bacter*	*Y. entero-colitica* O3	*S. flexneri* 2	*B. cereus*	Coinfections
0 – 24	87	52* (75.4)	14 (38.9)	18 (62.1)	4 (80.0)	0	1 (100)	2 (66.7)
25 – 60	42	14 (20.3)	20* (55.6)	7 (24.1)	1 (20.0)	1 (100)	0	1 (33.3)
> 60	9	3 (4.3)	2 (5.5)	4(13.8)	0	0	0	0
Total	141	69 (100)	36 (100)	29 (100)	5 (100)	1 (100)	1 (100)	3 (100)

* $p < 0.05$, χ^2; † RV – Rotavirus; ‡ microorganism

Table 5. Distribution of age groups according to isolated pathogens

Pathogens	Age Groups, N° (%)		
	0 – 24 Months	25 – 60 Months	> 60 Months
Rotavirus (n = 69)	52* (59.8)	14 (33.3)	3 (33.3)
Salmonella spp. (n = 36)	14 (16.1)	20* (47.6)	2 (22.2)
Campylobacter spp. (n = 29)	18 (20.7)	7 (16.7)	4 (44.5)
Y. enterocolitica O3 (n = 5)	4 (4.6)	1 (2.4)	0
S. flexneri 2 (n = 1)	0	1 (2.4)	0
Bacillus cereus (n = 1)	1 (1.1)	0	0
Coinfections (n = 3)	2 (2.3)	1 (2.4)	0
Total	87	42	9

*P<0.05, χ^2

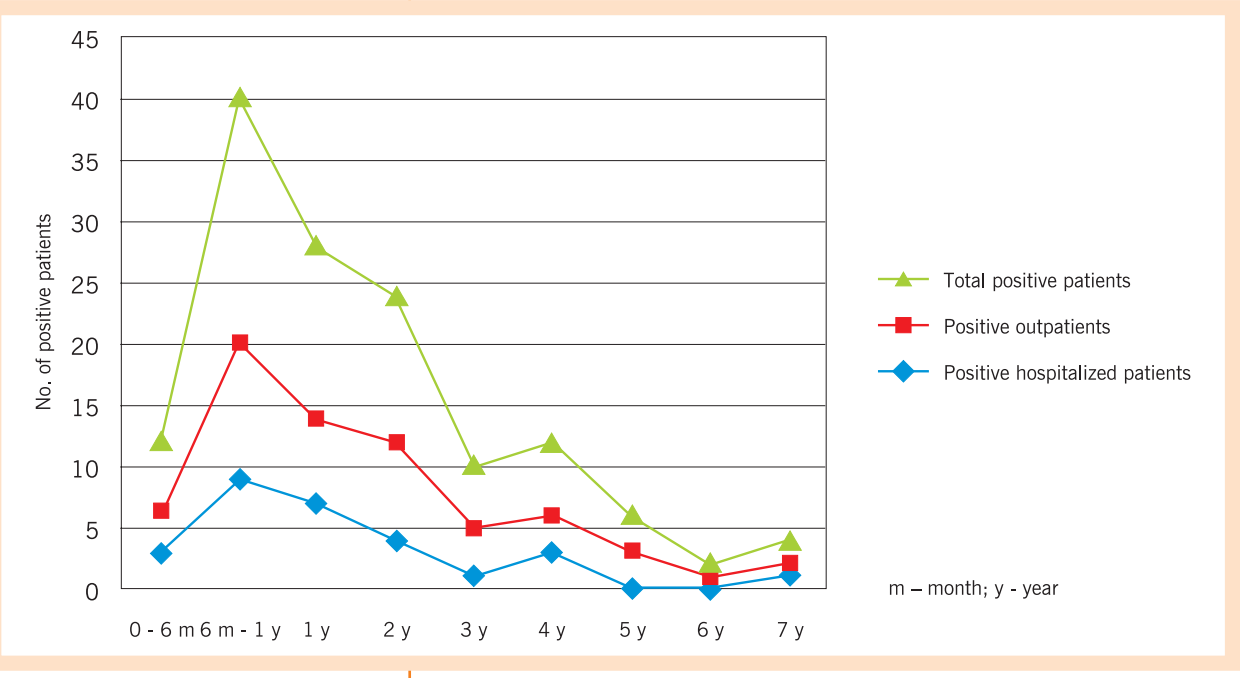


Figure 1. The presence of positive RVGE patient in different age groups

years of age, and in six (66.67%) cases, bacterial pathogens were detected, with *Campylobacter* spp. (n = 4; 44.5%) being the most common. In this group, RV was identified in three patients.

The highest incidence of total rotavirus infection was among the patients less than one year of age – 37.68% (26/69). Rotavirus infection was also diagnosed in 42.86% (12/28) hospitalized infants (0–1 year) with gastroenteritis (Figure 1). In all investigated children up to five years of age, the incidence of rotavirus infection was 95.65% (66/69).

RVGE was detected more frequent in males than in females.

RVGE was detected more frequent in males (41/69) than in females (28/69), as well as in hospitalized patients 17/28 (60.71%) compared to outpatients 11/28 (39.29%) (P = 0.1176). By the conventional criteria, this difference was not statistically significant. Of 1,156 children tested, non-viral pathogens were found in 6.23% (1156/72).

The most frequently isolated pathogens were *Campylobacter* spp. and *Salmonella Enteritidis* (group D). RV alone was detected in 46.48% (66/142) of positive findings, while non-viral pathogens alone were found in 49.30% (70/142). Non-viral GE etiology was represented in the following manner: 49.32% (36/73) by *Salmonella* spp., 39% (29/73) by *Campylobacter* spp., 6.85% (5/73) by *Yersinia enterocolitica* O3 (5/73) and 1.37% (1/73) by *Shigella* spp., *Bacillus cereus* and *Candida* sp. each (Table 7).

Mixed bacterial/viral infection was detected in 0.26% (3/1156) of investigated children (Table 6). In 139 GE patients with confirmed enteropathogens, mixed bacterial/viral infection was detected in 2.16% (3/139).

One of 69 positive hospitalized pediatric patients with RVGE had at the same time the infection caused by *Salmonella* group B (*S. Typhimurium*), and two outpatients were co-infected with *Campylobacter* spp.

Table 6. Etiologyof gastroenteritis in investigated patients 7 years of age and younger

Pathogen	No. (%) of children	
†RV alone	66	(5.71)
Mixed infection (RV and bacterial pathogen)	3	(0.26)
RV +*Salmonella* species	1	(0.087)
RV +*Shigella* species	0	(0.00)
RV + *Campylobacter* species	2	(0.173)
Non-viral enteropathogens alone	70	(6.05)
Not detected	1017	(87.98)

†RV – Rotavirus

Table 7. The frequency of isolated non-viral pathogens in children with *GE

Microorganism	No (%)
Salmonella	36 (49.32)
Salmonella group D *S. Enteritidis*	 29 (39.73)
Salmonella group B *S. Typhimurium* *S. Abony*	 5 (6.85) 1 (1.37)
Salmonella group C1 *S. Infantis*	 1 (1.37)
Campylobacter spp.	29 (39.73)
Yersinia enterocolitica O3	5 (6.85)
Shigella flexneri 2a	1 (1.37)
Bacillus cereus	1 (1.37)
Candida spp.	1 (1.37)
Total non-viral pathogens	73 (100)

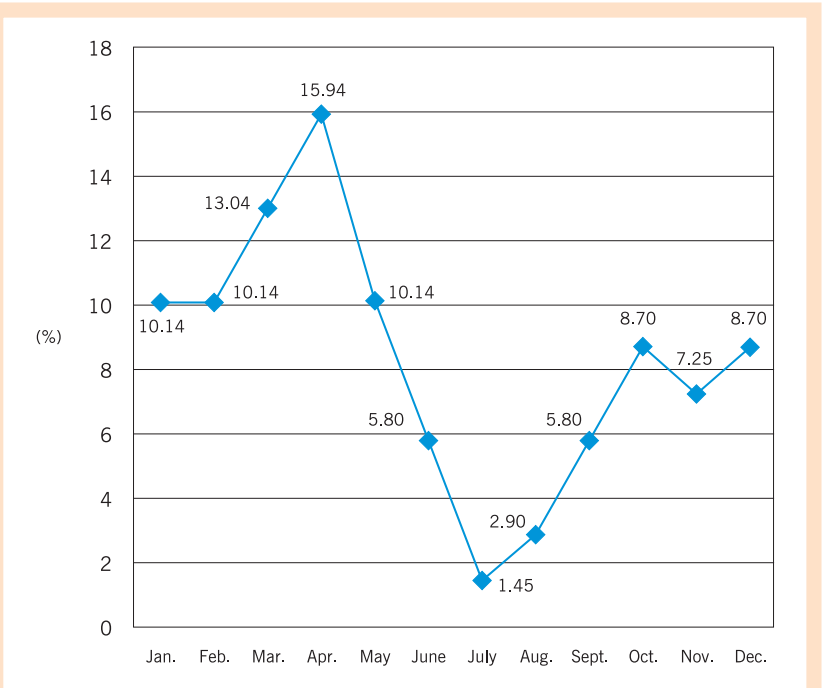
*GE – gastroenteritis

Table 8. Monthly distribution of bacterial and viral pathogens

Months	The number of isolated pathogens					
	Rotavirus	*Salmonella*	*Campylobacter*	*Y. enterocolitica*	*S. flexneri*	*B. cereus*
January	7	3	0	0	0	0
February	7	4	1	1	0	0
March	9	0	1	1	0	0
April	11	2	5	0	0	0
May	7	8*	4	1	0	1
June	4	4	2	0	0	0
July	1	6	5	1	0	0
August	2	1	0	0	0	0
September	4	4	4	0	1	0
October	6	2	2	0	0	0
November	5	1	3	0	0	0
December	6	1	2	1	0	0
Total	69	36	29	5	1	1

*P<0.05, χ^2

Figure 2.
The monthly prevalence of RVGE during the investigated period



Regarding the occurrence of RV infection in relation to season, the highest prevalence of GE was recorded in the winter/spring season, with its maximum in April.

Regarding the occurrence of RV infection in relation to season, the highest prevalence of GE was recorded in the winter/spring season, with its maximum in April (the two tailed P = 0.1825, by the conventional criteria, this difference was not statistically significant), n = 11; 15.94% (Table 8 and Figure 2, 3). A decrease in RVGE occurred in the summer, with the lowest incidence in July (1.45%) (Figure 2). A statistically significant difference was found in the occurrence of rotavirus infection between summer months and other seasons of the year (Chi-square test). The two-tailed P value equals

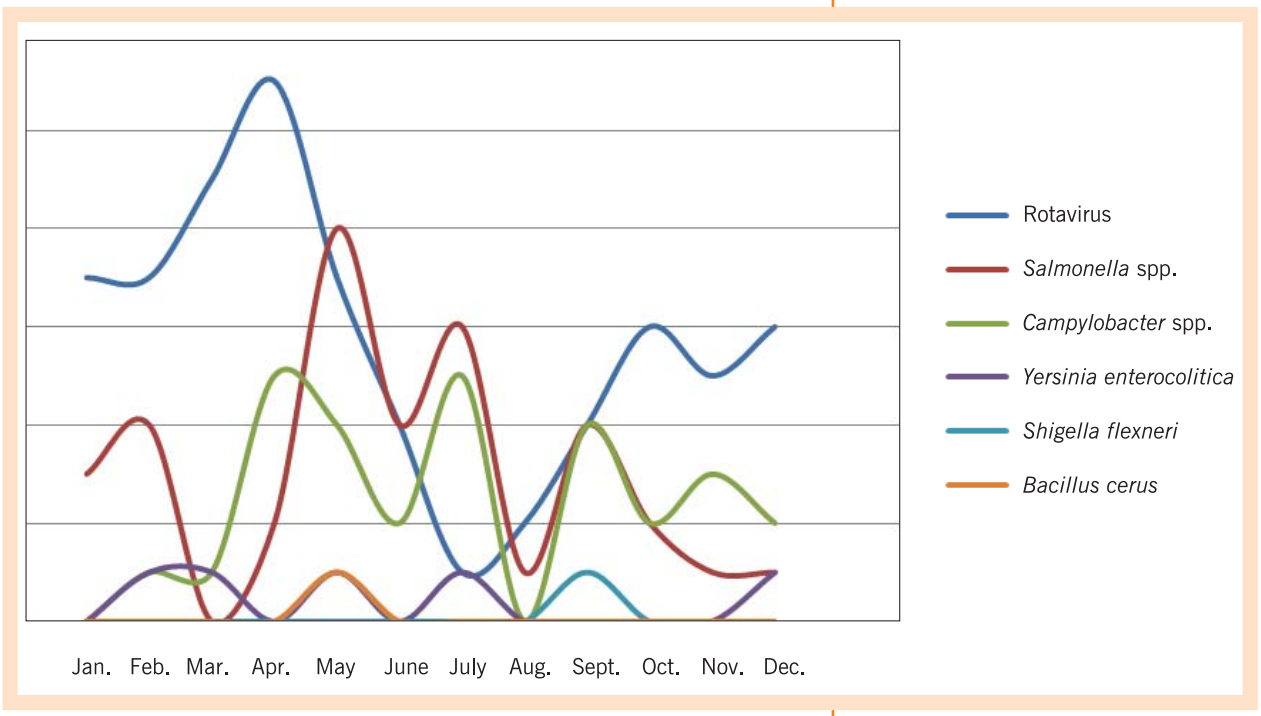


Figure 3.
Seasonal distribution of acute gastroenteritis agents

0.0063 ($p < 0.01$). By the conventional criteria, this difference was considered to be highly statistically significant. The peak number of *Salmonella* spp. cases, which occurred in May (n = 8; 22.22%), was statistically significant (the two tailed P = 0.0453), while there was not any cases in March (Figure 3).

DISCUSSION

Acute GE is one of the most common infectious diseases with variable etiology depending on the age, demographic features and seasonality in both developed and developing countries. In addition to the bacterial causes such as *Salmonella* spp., *C. jejuni*, *Shigella* spp., viruses (rotavirus, norovirus, adenovirus, astrovirus) have been reported as etiological agents. It is thought that viruses affect all age groups and are responsible for the majority of GE cases [16, 17, 18]. Among the patients with diarrheal illness, there is a large group with indeterminate etiology, and therefore an accurate diagnosis is required for an effective treatment and prognosis.

Since we examine samples from clinic and outpatients, but not patients in emergency unit, it is possible that it could influence the positivity of the obtained results. Moreover, RV vaccine is not part of the vaccination policy in Serbia.

In this study, pathogens were detected in 11.42% stool samples from 1,156 children under eight years of age, with acute GE. However, the most frequent pathogen detected was rotavirus (12.7%). Other viruses were not searched for. Bacterial enteric pathogens of acute gastroenteritis included *Salmonella* spp., *Campylobacter* spp., *Yersinia enterocolitica* O3, *Shigella flexneri*, *Bacillus cereus*, with detection rates of 6.23%.

In this study, the most frequent pathogen detected was rotavirus.

At the pediatric emergency clinic of a university hospital in Istanbul, during 12 months of observation, rotavirus was the most frequent pathogen, with a rate of 12.7% (75/588) [19]. In Papua, New Guinea, rotavirus (25.6%) and adenovirus (11.6%) were the enteric pathogens most commonly detected [20]. In children attending day care who experienced substantial gastrointestinal infections due to circulating seasonal enteropathogens in the day care environment in the Netherlands in 2010–2013, rotavirus was detected in 11%, norovirus in 10% and astrovirus in 7%. The authors demonstrated that circulating viruses, rather than bacteria, contribute to seasonal gastroenteritis experienced by children in day care [21].

In this study, RVGE was detected more frequently in hospitalized patients than in outpatients and the most affected group included infants aged six months to one year of age. RVGE was reported in 32.15% of hospitalized children, in 26.82% of outpatient children, and overall in 28.98% (Table 3). It appeared that this group of patients comprised the mayor group seeking medical attention. The highest rate of RV infection has been reported in patients 6 to 24 months of age [8] in both developed and non-industrialized countries. Concerning the RV positive cases, 28.0% were younger than 24 months, 57.3% were between 25 – 60 months, and 85.3% were younger than five years in Pediatric Emergency Unit of Yeditepe University Hospital, Istanbul, Turkey [19]. In Papua, New Guinea, an analysis of the age distribution of infections between the children aged < 1 year and children aged 1–5 years showed no significant difference in the detection rate of pathogens between the two age groups.Young children were much more likely to be admitted to hospital with acute gastroenteritis than older children [20]. In Palermo, Sicilia (Italy), the mean and median ages were 46.3 and 25.8 months, respectively (range, 4–163.6 months) [22].

RVGE was detected more frequently in hospitalized patients than in outpatients and the most affected group included infants aged six months to one year of age.

The overall gender distribution of 69 RV positive children revealed the proportion of 59.42% (41/69) for male and 40.58% (28/69) for female patients. RVGE was detected more frequently in males than in females, as in 17/28 (60.71%) hospitalized and in 41/69 (59.42%) outpatients, without statistical significance. We could conclude that gender does not seem to influence the susceptibility to RV similarly to the previous studies [20, 22, 23].

In this study of 1,156 hospitalized patients and outpatients, the most frequently isolated bacterial pathogens were *Salmonella* spp.: *S. Enteritidis* (29), *S. Typhimurium* (5), *S. Abony* (1), *S. Infantis* (1) *Campylobacter* spp. (29) and *Yersinia enterocolitica* O3 (5). The ratio viral *vs* bacterial infections was almost the same 69:72. The review of clinical records of patients with an acute GE, aged less than 15 years presenting to the pediatric emergency department of a Portuguese district hospital over a 30-month period, revealed that of 216 stool samples, 98 (45%) were positive: *Campylobacter* spp. was identified in 50%, *Salmonella* spp. in 28%, *Yersinia enterocolitica* in 10%, *Aeromonas* spp. in 9% and *Escherichia coli* O157 in 3% [24]. At the University hospital in Istanbul, Turkey, bacterial enteric pathogens of

acute GE were *Salmonella* spp. and *C. jejuni*, with the detection rates of 25.6% (21/82) and 18.3% (15/82), respectively [19]. In a one-year investigation of hospitalized patients in Palermo, Sicily, Italy, the bacterial pathogen was detected in 127 (59.1%) cases. Monobacterial infections were detected in 26 (12.1%) patients (*Salmonella* spp. in 18, *Shigella* spp. in 3, *C. jejuni* in 5 pts), while single viral infections were found in 80 (37.2%) patients [22]. In Papua, New Guinea, *Shigella* spp. were the bacterial pathogens most commonly detected (26.6%) [20]. EPEC strains were detected in 8.5% of patients. ETEC strains were detected in 11.1% of the samples. *Campylobacter* spp. was detected in 4.0%, while *Salmonella* spp. occurred sporadically. *V. cholerae* was not detected in any patient. Our results were more similar to the results obtained in Mediterranean Basin (part of Istanbul, Palermo) than in New Guinea in Oceania (the eastern half of the island of New Guinea and islands in Melanesia) a region north of Australia. Climate, geographic, demographic and other factors might influence the differences in gut microbiota.

The indistinguishable clinical presentation of gastroenteritis in children, together with the diversity of enteropathogens, makes the assessment of individual contributions of enteropathogenic agents that may cause gastroenteritis more challenging.

The indistinguishable clinical presentation of gastroenteritis in children, together with the diversity of enteropathogens, makes the assessment of individual contributions of enteropathogenic agents that may cause gastroenteritis more challenging.

Mixed infections were detected at a high frequency (22.1%) in Papua, New Guinea, (20.7%) in Tanzania, as well as in other developing settings such as Jordan (15.5%), Libya (13.8%), Vietnam (13.5%), and Brazil (11.0%). On the contrary, co-infections are typically reported at lower frequency in the developed milieu such as Italy (9.8%), Denmark (1.9%), and France (1.1%), as in our country (2.16%). These findings might reflect low levels of sanitation and hygiene in the developing countries [20].

We have found 0.26% of RV mixed infections of total investigated children and in 2.21% of those with positive findings. This result was similar to those reported in UK (2%) [25] and other developed countries. The detected co-infections were RV and *Salmonella* spp. in one patient and RV with *Campylobacter* spp. in two patients. In Papua, New Guinea, the combination of the pathogens *Shigella* (13.1%) and rotavirus (11.1%) was most commonly detected in mixed infections [20]. At the pediatric emergency of a University hospital in Istanbul during 2009, in 184 positive stool samples only two cases of coinfection with bacteria-virus were recorded: in one case rotavirus, adenovirus and *Salmonella* spp., and in the other case adenovirus and *Salmonella* spp. [19]. In the study in Sicily in hospitalized patients, viral bacterial coinfection was present in 4.6% cases, viral/viral coinfection in further 4.6%, and double-viral and bacterial coinfection in 0.5% cases [22]. Mixed bacterial viral infection presents more severe clinical manifestation, changing the gut microbiota as well [26].

RVGE is considered to be a relevant cause of peditric nosocomial diarrhhea as well. The overall incidence of RVGE in hospitalized children

Rotaviruses are the substantial factor in the etiology of acute diarrheal diseases in the town of Nis, Serbia, and the surrounding area, especially among hospitalized patients in winter and early spring season.

in Europe is from 31% to 87% of nosocomial diarrhea [27]. One investigation in Poland (2006–2010) revealed that the mean proportion of nosocomial RVGE among all rotavirus infections was 24%, with the highest rates in children younger than two years [27, 28]. In the investigated period, we did not find any nosocomial infections caused by RV in hospitalized children.

In a Polish study, 69% of rotavirus infections occurred in the autumn and winter season [29], which is similar to other data obtained for the temperate or subtropical regions in developed countries [25, 27]. However, our data indicated the highest rate of RGVE in winter/spring season, with its maximum in April. In Turkey, RV infection was most frequent in the period January – April [19]. Rotavirus cases may vary by geographical location: in temperate climates most cases are observed in fall, winter and spring months [11], while in tropical countries there are not seasonal peaks [12]. However, in Burkina Faso among the children under five years of age, rotavirus infections occur more often in the dry season when compared to the wet season ($p = 0.03$) [30].

Although viral etiology of GE can be recognized using numerous simple and fast tests based on ELISA and immunchromatography, due to which unnecessary antibiotic use is thus avoided, molecular PCR-based techniques can be used to improve the overall diagnostic efficacy of viral and bacterial GE and to detect types and subtypes, which should promote various types of studies.

CONCLUSION

Although our study was not a case–control study, our investigation provided some important data on the presence of enteric pathogens in children presenting with GE. Our data of RVGE in children, during this 18-month research period, indicate that rotaviruses are the substantial factor in the etiology of acute diarrheal diseases in the town of Nis, Serbia, and the surrounding area, especially among hospitalized patients in winter and early spring season. In addition to RVGE, *Salmonella* spp. and *Campylobacter* spp. were the major contributors to childhood diarrheal illnesses in our setting.

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