Journal of Applied Animal Research

ISSN: 0971-2119 (Print) 0974-1844 (Online) Journal homepage: https://www.tandfonline.com/loi/taar20

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To cite this article: Conrado Carrascosa, Esther Sanjuán, Rafael Millán, Sara Martín, Pedro Saavedra, António Raposo, Cristóbal del Rosario-Quintana & José Raduán Jaber (2019) Is the use of supermarket trolleys microbiologically safe? Study of microbiological contamination, Journal of Applied Animal Research, 47:1, 17-23, DOI: 10.1080/09712119.2018.1555091

To link to this article: https://doi.org/10.1080/09712119.2018.1555091

Published online: 12 Dec 2018.

JOURNAL OF APPLIED ANIMAL RESEARCH
2019, VOL. 47, NO. 1, 17–23
https://doi.org/10.1080/09712119.2018.1555091

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Is the use of supermarket trolleys microbiologically safe? Study of microbiological contamination

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ABSTRACT

Microbial contamination in shopping trolleys (eighty five) by considering different supermarkets (seven) from three major food companies in Las Palmas de Gran Canaria (Spain) was determined.

The two sampled areas were trolley handles and food trolley baskets-child seats. Samples were analyzed by selective and differential microbiological culture media.

E. coli four (2.4%) indicative of faecal contamination, *Klebsiella pneumoniae* twelve (6.5%) and *Citrobacter freundii*, six (5.1%), which have been isolated from human faecal samples, were isolated from trolleys; *Pseudomonas rhodesiae*, five (4.25%), and *Pseudomonas fluorescens*, three (2.55%), which both evidenced environmental contamination. Significant differences among the companies were found for the Enterobacteriaceae and coliforms. Regarding location, these differences ($p < 0.003$) were observed only for the coliform rates, which were higher in trolleys located outside.

The results of this study suggest the implementation of cleaning and disinfection programmes to improve trolley sanitation, and to reduce exposure to both potential pathogenic and transmitting bacterial infections.

ARTICLE HISTORY
Received 1 August 2018
Accepted 27 November 2018

KEYWORDS
Food baskets-child seats; handles; microbiological contamination; shopping trolleys; surfaces

Introduction

Inanimate objects (fomites) for public use, such as shopping trolley handles, lift buttons, handrails, etc, which come into direct contact with users' hands, are a source of contamination of potential pathogenic microorganisms. They come into contact either directly by surface-to-mouth contact or indirectly by contaminated fingers and subsequent hand-to-mouth contact (Gerba and Maxwell 2012; Irshaid et al. 2014). Some studies have reported frequent exposure to pathogenic *Staphylococcus aureus* on shopping trolley handles, suggest that it is a hidden reservoir of this organism, and indicate a shopping basket/trolley sanitation necessity (Mizumachi et al. 2011). Shopping trolley contamination may occur from directly handling raw food products or trolleys contaminated by previous users (Gerba and Maxwell 2012). Nevertheless, cross-contamination in shopping trolley baskets occurs when disease-causing microorganisms are transferred from one food type to surfaces or, as in this case study, when dirty hands transfer microorganisms to trolley handles or baskets. For example, raw meat products are often contaminated with foodborne bacteria, such as *Salmonella* and *Campylobacter* (Bier et al. 2004), which may be transferred to surfaces. However, the level of bacterial contamination on shopping trolleys or shopping baskets is limiting making health assessment difficult (Mizumachi et al. 2011). It is believed that up to 80% of common infections can be spread through coming into contact with contaminated surfaces (Reynolds et al. 2005). Pathogenic organisms, i.e. viruses, bacteria and protozoa, may be excreted in large numbers in biological substances, including blood, mucus, saliva, faeces and urine (Hall and Douglas 1981; Hall et al. 1981; Feachem et al. 1983; Uhnoo et al. 1990; Weber et al. 1994; Islam et al. 2001). Some microbes are infectious at very low concentrations and can survive on different surfaces like countertops and telephone handpieces for hours, and even for weeks (Noskin et al. 1995; Bures et al. 2000; Abad et al. 2001).

Hands are frequently involved in such episodes, and act as vehicles that spread infections in both the community and hospitals (Pittet et al. 2000). Several studies have been published about microorganism contagion from contaminated fomites with pathogens to healthcare workers' hands in hospitals (Kramer et al. 2006; Mizumachi et al. 2011). However, a correlation between the burden of contamination on hands and the likelihood of transmission to patients has not yet been established (Bellissimo-Rodrigues et al. 2017). Nonetheless, common infectious diseases still feature among the top 10 leading causes of death (Liu et al. 2012; Willmott et al. 2016), and healthcare-associated infections are recognized as a major cause of preventable death in healthcare settings (Willmott et al. 2016).

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Regarding bacterial persistence on surfaces, enterococci species are able to survive for 24 h with no significant reduction in colony counts. Most gram-positive bacteria, such as *Enterococcus* spp. (including VRE), *S. aureus* (including MRSA) or *Streptococcus pyogenes*, survive for months on dry surfaces (Kramer et al. 2006). Many gram-negative species can also survive for months, such as *Acinetobacter* spp., *Escherichia coli*, *Klebsiella* spp., *Pseudomonas aeruginosa*, *Serratia marcescens* or *Shigella* spp. A few others, like *Bordetella pertussis*, *Haemophilus influenzae*, *Proteus vulgaris* or *Vibrio cholerae*, persist only for days (Kramer et al. 2006).

Interestingly, vancomycin-resistant enterococci are capable of prolonged survival on hands, gloves and environmental surfaces, they persist for 60 min on telephone handpieces and for 30 min on the diaphragmatic surface of a stethoscope (Noskin, et al. 1995). Other authors (Wade et al. 1991) have documented the survival of vancomycin-resistant *E. faecium* on hands for up to 30 min and bacteria such as *S. aureus*, *Acinetobacter*, *Klebsiella aerogenes*, *Escherichia coli*, *Serratia marcescens*, and *Pseudomonas aeruginosa* (Casewell and Desai 1983). Hence hand washing is considered an essential aspect of infection control to prevent microorganisms from being transmitted. A 30-second wash with soap and water is necessary to completely eradicate bacteria from hands (Noskin et al. 1995).

The increased availability of shopping trolleys in supermarkets, handled by numerous users on a daily basis, and the fact that trolleys are not routinely disinfected, are a potentially excellent opportunity for contaminating microorganisms to be transmitted (Anderson and Palombo 2009). This is particularly true for infants and children under the age of 5 years with reported 2- to 10-fold higher risk rates than for people aged 5 years or more (Jones et al. 2006; Ailes et al. 2008).

Riding infants in a shopping trolley next to packaged raw meat and poultry has been shown to be an important risk factor for *Salmonella* spp. and *Campylobacter* spp. infection, with attributable risks of 11% and 7%, respectively (Jones et al. 2006; Fullerton et al. 2007; Patrick et al. 2010) as these microorganism have been isolated from outer packages of meat and poultry products at retail outlets (Harrison et al. 2001; Wong et al. 2004; Burgess et al. 2005).

The aim of this study was to determine the microbial contamination and bacterial species on shopping trolley handles and baskets in different supermarkets on the Gran Canaria Island (Spain). In addition, the statistic relationship between metal and plastic shopping trolleys microbiological contamination was studied, as was the location of trolleys, either outside or inside supermarkets.

Material and methods

Sampling was performed in three different supermarket companies of Las Palmas de Gran Canaria (A, B, C), the Canary Islands (Spain), for 3 months. Eighty-five shopping trolleys from seven supermarkets were sampled to determine the microbiological contamination on trolleys. We collected 85 swabs from trolley handles and 83 other swabs from the food baskets-child seats (basket-seat). The latter samples were considered from a single site (Figure 1).

Sampling the contact surfaces of trolleys

To this end, a maximum 100-cm^2 area was sampled per swab. Shopping trolleys were sampled for microbial contamination, and were subsequently tested for the qualitative analyses of pathogenics (Gerba and Maxwell 2012). A sterile rayon-tipped swab (Copan Flock Technologies Srl., Brescia, Italy), moistened with sterile saline solution (preservative-free), was moved over the entire surface of trolley handles and a new swap was used over baskets-seats. Swabs were aseptically transferred to a tube that contained 10 ml of sterile 0.1% peptone water (adapted from Salo, et al. 2002), and were delivered 2 h after being packed on ice to be sent to the Hygiene Laboratory of Veterinary Faculty of Las Palmas de Gran Canaria University, where samples were processed before 24 h had elapsed.

The total number of shopping trolleys from each sampled company was determined by a statistical stratified analysis, where the following were sampled: 35 (2,700 in all) shopping trolleys in one supermarket of company A; 30 (2,200 in all) shopping trolleys in three supermarkets of company B; 20 (800 in all) shopping trolleys in three supermarkets of company C.

Microbiological analysis and identification

Decimal dilutions in peptone water solution (0.85% NaCl with 0.1% peptone; Cultimed, Barcelona, Spain) were used for microbial enumeration purposes. Tubes were shaken vigorously. Appropriate dilutions were prepared by using sterile 0.1% peptone water and were plated by the pour plate method on different bacteria selection agar (Figure 1). After incubating the plate, the morphological characteristics of microorganisms were associated with each growth medium. Data are reported as colony-forming units (CFU/cm^2). All the counts were taken in duplicate.

Total viable counts (TVCs) and mesophilic bacteria were determined using Plate Count Agar (PCA Cultimed, 413799), and were incubated at 31°C for 72 h (Pascual and Calderón 2002; Broekaert et al. 2011). Enterobacteriaceae were determined using Violet Red Bile Glucose Agar (VRBG), (Cultimed, 413745, Barcelona Spain). Incubation was done at 37°C for 24 h. Bacteria were represented as large colonies with purple haloes, as described by other authors (Pascual and Calderón 2002).

S. aureus was isolated by Baird Parker+Rabbit Plasma Fibronogen agar (bioMerieux, Marcy ÍEtoile, France; ISO 6888–2; ISO, 1999), and was incubated at 37°C for 24–48 h. Escherichia coli was identified by ChromID coli® (bioMerieux; AFNOR, 2014) and was incubated at 37°C for 24–48 h.

E. faecalis was determined in kanamycin-esculin-azide broth (KAA) (Canamicina Esculina Azida, Cultimed, 464695.0922), and was incubated at 35°C for 48 h following the manufacturer's instructions. Positive growth was considered if the tube changed to blackish-green. *E. faecalis* was spread on KAA agar and incubated at 35°C for 24–48 h. Finally, grown colonies were confirmed as *E. faecalis* when they were gram-positive cocci with a negative catalase test, and were able to grow on bile esculin agar (BEA) incubated at 42°C by esculin hydrolysis (Greenberg et al. 1992; Dionisio and Borrego 1995).

The microorganisms isolated from VRBG plates were identified using gram stain, colony counts, morphology, and catalase and

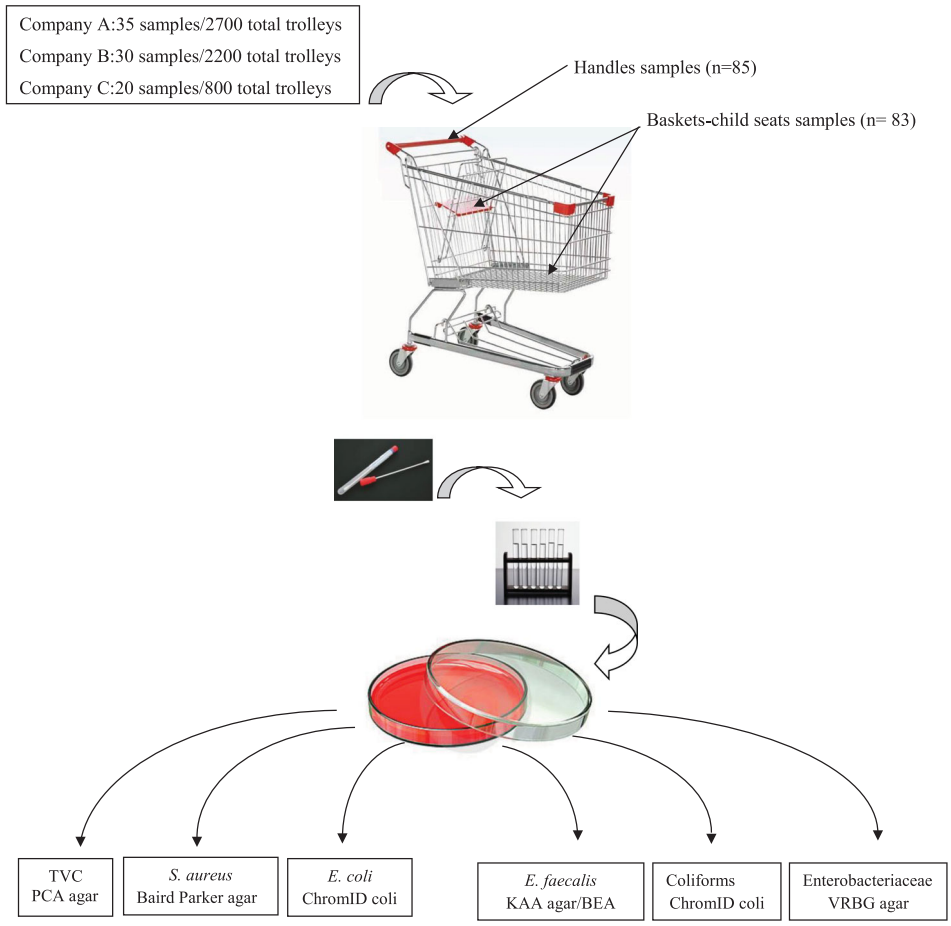

Figure 1. Flowchart of sampling in the trolleys.

oxidase reactions. Gram-negative bacteria were identified by API 20 E and mass spectrometry in a Bruker Biotyper matrix-assisted laser desorption ionisation-time of flight mass spectrometry (MALDITOF MS) system (Bruker Daltonics, Germany) with a higher score level than 2,200 for species identification.

The Analytical Profile Index (API) 20 E test kit (BioMerieux®, Marcy ÍEtoile, France) was used following the manufacturer's instructions. Strips were examined after 24 and 48 h. Isolates were identified according to the API 20 E identification online instructions (http://apiweb.biomerieux.com).

There are no standards available for fomites surface counts. However, a general microbial target value of <2.5 CFU/cm^2 after disinfection has been found to be attainable for a range of surfaces in food industries (Carrascosa et al. 2012). The preliminary data obtained in this study showed that the total viable counts in non-disinfected objects were considerably higher. Even so, were obtained an average of 753 CFU/cm^2, a minimum of 80 CFU/cm^2 and a maximum of 18,700 CFU/cm^2.

Table 1. Contamination rates of surfaces at different supermarket companies (A, B, C) locations and shopping trolley materials.

Factor	Microorganism	Level	*n* / total (%)*	*P*-value	OR (95%CI)
Company	Enterobacteria (1)	A	22/35 (62.9)[a]	.003	1
		B	7/30 (23.3)[b]		0.180 (0.061–0.534)
		C	6/20 (30.0)[b]		0.253 (0.078–0.821)
	Coliforms (1)	A	14/25 (56.0)[a]	.013	1
		B	6/30 (20.0)[b]		0.196 (0.060–0.648)
		C	2/10 (20.0)[a,b]		0.196 (0.035–1.118)
	Enterobacteria (2)	A	24/35 (68.6)[a]	.008	1
		B	9/30 (30.0)[b]		0.196 (0.068–0.566)
		C	9/18 (50.0)[a,b]		0.458 (0.143–1.473)
	Coliforms (2)	A	15/25 (60.0)[a]	.028	1
		B	10/30 (33.3)[b]		0.333 (0.111–1.004)
		C	7/9 (77.8)[a]		2.333 (0.400–13.609)

(*) Distinct superscripts ([a, b, c]) indicate significant differences for $p < .05$.
Surface sampled: 1, handles; 2, food baskets-child seats.

Statistical analysis

The contamination rates for the considered microorganisms were summarized as frequencies and percentages, and were compared by the Chi-square (χ^2) or the exact Fisher test whenever appropriate. Odd ratios by means of 95% confidence intervals (95%CI) were used for the comparisons that showed statistical significance. Statistical significance was set at $p < .05$. Data were analyzed with the R package, version 3.3.1 (R Development Core Team 2016).

Results

Eighty-five shopping trolleys were sampled, and the enteric bacteria species on handles and baskets-child seats were isolated. Shopping trolley handles were found to be contaminated by enterobacteria on thirty five (41.17%) surfaces and on forty three (50.6%) baskets-child seats. Coliforms were growing on handles on twenty two (25.9%) trolleys and on thirty nine (45.9%) baskets-child seats. *E. coli* was identified only on three (2.55%) basket-child seats. Neither *S. aureus* nor *E. faecalis* was detected by specific agar medium.

Table 1 contains the contamination rates of the sampled surfaces of the different supermarket companies (A, B, C). This table also summarizes the contamination rates according to supermarket companies, location and material (Table 2). Company B showed significantly lower contamination rates than those obtained for Company A for enterobacteria on handles (23.3% *vs.* 62.9%), coliforms on handles (20% *vs.* 56%), enterobacteria on baskets (30% *vs.* 68.6%) and coliforms on baskets (33.3% *vs.* 60%). All the results obtained from Companies A, B and C showed significant differences for the coliforms and enterobacteria rates on both surfaces. *E. coli* on baskets was detected only on one Company A trolley, whereas *S. aureus* and *E. faecalis* were not detected on any analyzed trolley from companies A, B, C.

For the relationship of the location (outside or inside) of shopping trolleys in supermarkets and their contamination, we found significant differences, but only for the coliforms rates (56.0% *vs.* 20.0%, $p = .003$) on handle surfaces. Contamination was higher outside than it was inside (Table 2). Likewise, the comparison made of the plastic and metal material used to manufacture shopping trolleys showed no significant differences (Table 2). The highest contamination rates on handles and on basket-child seats were on plastic material, except for coliforms contamination on handles, which was higher for metal trolleys (Table 2).

In addition, *E. coli* and other potential pathogenic bacteria were also isolated from both surfaces, but showed different rates. The isolations of the bacteria that belonged to the Enterobactericeae family were particularly interesting, including *Klebsiella pneumoniae* isolated from 11 (6.5%) shopping trolleys, and *Citrobacter freundii* isolated from 6 (5.1%), which may be found in human faeces. *Pseudomonas rhodesiae* and *P. fluorescens* were isolated from five (4.25%) and three (2.55%) shopping trolleys, respectively, and these bacteria evidenced environmental contamination. Table 3 shows other bacteria of special interest that were isolated from trolleys.

Table 2. Contamination rates of surfaces at different locations and shopping trolley materials.

Factor	Microorganism	Level	*n* / total (%)*	*P*-value	OR (95%CI)
Location	Enterobacteria (1)	Inside	13/40 (32.5)	.125	1
		Outside	22/45 (48.9)		1.987 (0.822–4.803)
	Coliforms (1)	Inside	8/40 (20.0)	.003	1
		Outside	14/25 (56.0)		5.091 (1.684–15.390)
	Enterobacteria (2)	Inside	16/39 (41.0)	.100	1
		Outside	26/44 (59.1)		2.076 (0.864–4.989)
	Coliforms (2)	Inside	17/39 (43.6)	.200	1
		Outside	15/25 (60.0)		1.941 (0.700–5.384)
Material	Enterobacteria (1)	Metal	7/24 (29.2)	.158	1
		Plastic	28/61 (45.9)		2.061 (0.747–5.681)
	Coliforms (1)	Metal	6/14 (42.9)	.527	1
		Plastic	16/51 (31.4)		0.610 (0.181–2.049)
	Enterobacteria (2)	Metal	9/22 (40.9)	.289	1
		Plastic	33/61 (54.1)		1.702 (0.634–4.572)
	Coliforms (2)	Metal	6/13 (46.2)	.756	1
		Plastic	26/51 (51.0)		1.213 (0.358–4.113)

(*) Distinct superscripts ($^{a, b, c}$) indicate significant differences for $p < .05$.
Surface sampled: 1, handles; 2, food baskets-child seats.

Discussion

Microbiological counts in the analyzed shopping trolleys from different supermarkets showed a high contamination rate on both sampled surfaces, which was slightly higher on baskets-child seats. Most studies about fomites contamination have been undertaken on hospital equipment surfaces (Kramer et al. 2006; Bellissimo-Rodrigues et al. 2017), but very few studies have determined contaminated shopping trolleys (Al-Ghamdi et al. 2011; Ashgar and El-Said 2012; Gerba and Maxwell 2012; Irshaid et al. 2014), which indicates that consumers are exposed to enteric bacteria from grocery shopping trolleys on a regular basis. In our study, total bacterial levels were far higher than those found in public restrooms and other public places (airports, bus stations, public bathroom, shopping malls, etc.) reported by other authors (Gerba and Maxwell 2012). Those studies had sampled different surfaces with the same swab (handles-child seat) and obtained a single result for all the surfaces.

In the present study, we found up to 45.9% of coliforms and 2.45% of *E. coli*, whereas Gerba and Maxwell (2012) found higher rates (72% and 21.17%, respectively). Similar results were shown by Reynolds et al. (2005) and Al-Ghamdi et al. (2011) and who determined 20% of coliform and 7% of faecal coliform contamination, respectively. Regarding contamination sources, Reynolds et al. (2005) found no relationship to link biochemical markers, protein and bacterial contamination on public surfaces, including shopping trolley handles. While the presence of biochemical markers and protein provides

Table 3. Relation of the bacteria of special interest isolated from trolleys identified with MALDITOF.

Isolated Bacteria	*n* / total (%)
E. coli	4 (2.4)
Klebsiela pneumoniae	11 (9.3)
Citrobacter freundi	9 (5.3)
Pseudomonas rhodesiae	4 (2.3)
Pseudomonas fluorescens	3 (1.7)
Enterococcus faecalis	3 (1.7)
Staphylococcus haemolyticus	3 (1,7)
Streptococcus gallolyticus	3 (1.7)
Morganella morganii	2 (1.2)
Proteus mirabilis	2 (1.2)
Enterobacter asburiae	2 (1.2)

information on the relative hygiene of various environments, very little is known about their correlation with infectious microbes (Reynolds et al. 2005). Nonetheless, other authors have described longer microbial persistence with higher inoculum in the presence of protein (Neely 2000), serum (Elmos 1977; Hirai 1991) or without dust (Wagenvoort and Penders 1997), but these studies were undertaken on fomites in hospitals.

Thus persistence of bacteria on surfaces can vary subject to intrinsic factors from microorganisms: gram-negative bacteria have been described to persist longer than gram-positive bacteria (Dickgiesser 1978; Hirai 1991). The latter are transmitted readily from environmental surfaces, followed by viruses and gram-negative bacteria (Rusin et al. 2002). Humid conditions and low temperatures, e.g. 4°C or 6°C, also improve the persistence of most bacteria types, such as *Listeria monocytogenes* (Helke and Wong 1994), *Salmonella typhimurium* (Helke and Wong 1994), Methicillin-resistant *Staphylococcus aureus* (MRSA) (Noyce et al. 2006), or *Escherichia coli* (Wilks et al. 2005; Williams et al. 2005). These risks can be minimized by applying a shopping trolley cleaning and disinfection programme using antimicrobial agents, according to the data reported in this field (Rutala and Weber 2001; Engelhart et al. 2002), and by devising efficient planning to rotate the periodical cleaning of all supermarket trolleys (between 800 and 2,700 trolleys per supermarket).

The potential pathological microorganism findings on the shopping trolleys included in this study agree with those reported by other authors (Reynolds et al. 2005; Al-Ghamdi et al. 2011; Ashgar and El-Said 2012; Gerba and Maxwell 2012). Our results revealed the importance of cleaning and disinfecting shopping trolleys to avoid the presence of *K.* pneumonia, which is an opportunistic pathogen responsible for a high proportion (4–8%) of nosocomial infections (Podschun and Ullmann 1998).

In addition, many studies have clearly shown that *E. coli* is the only coliform that is an undoubted inhabitant of the gastrointestinal tract. While *Klebsiella* spp., *Citrobacter freundii* and Enterobacter have been isolated from human faecal samples, they are in small numbers when present (Edberg et al. 2000). However, these opportunistic pathogens isolated herein such as *C. freundii*, which can cause systemic infections (Kim et al. 2003; Pereira et al. 2010; Chen et al. 2011). Moreover, *C. freundii* has been used to control good healthy measures, like periodical cleaning of tools and abattoir surfaces (Milhem et al. 2016). *P. fluorescens* has been reported to cause infections like blood transfusion-related septicaemia (Khabbaz et al. 1984). Thus, results of epidemiological studies have shown that a risk of infection from common enteric bacteria is related to the placing of small children in shopping carts (Fullerton et al. 2007; Patrick et al. 2010) and it increases the risk of coming into contact with a disease-causing organism.

When comparing plastic or metal shopping trolleys, plastic ones showed higher contamination, but differences were not significant. Nevertheless, larger sample sizes should be considered to obtain significant differences ($p < 0.05$). the tested material types gave no consistent results for nosocomial persistent pathogenics on inanimate surfaces. Although some researchers have reported that this type of material has no influence on persistence (Bale et al. 1993; Wendt et al. 1997), other authors have described longer persistence on plastic (Neely and Maley 2000). This topic can be clearly observed in food industries, where the ability of many bacteria to adhere to surfaces and to form biofilms has major implications. Properties like surface roughness, cleanability, disinfectability, wettability and vulnerability to wear influence the ability of cells to adhere to a particular surface, and thus determine the hygienic status of materials (Van Houdt and Michiels 2010). Other authors (Bellissimo-Rodrigues et al. 2017) have described a direct relationship between the bacterial load present on hands and the risk of cross transmission following a single hand-to-hand contact. Under the described experimental conditions, at least 1 $\log_{10}$ CFU of *E. coli* must be present on hands for it to be potentially transmitted to another person. This threshold may be useful to develop an evidence-based 'safe hands' microbiological concept that can be applied in the healthcare setting, and in the general community to prevent infections and antimicrobial resistance from spreading (Bellissimo-Rodrigues et al. 2017).

Conclusions

The obtained results suggest the need to establish adequate cleaning and disinfection programmes for shopping trolleys in order to avoid exposing shopping trolley users to infections. The most effective measure could be the use of an alkaline detergent and quaternary ammonium as a disinfectant, washing the shopping trolleys once a month and doing a proper rotation of them. The material (metal or plastic) and location (outside or inside) of the shopping trolleys in the supermarkets should be taken into consideration during the preparation of the cleaning and disinfection plan, to reduce microbial contamination more effectively. As additional measures, some studies have proposed using disinfecting wipes or disposable plastic barriers for handles. In the future, we hope to compare our contamination results and potential pathogenic rates with other studies done on supermarket trolleys, which have established a cleaning and disinfection programme to assess their efficacy and to finally answer the age-old debate about the relevance of environmental contamination.

Highlights

- Determination of microbial contamination in shopping trolleys located in Las Palmas.
- Isolation and identification of several pathogenic bacteria.
- Environmental contamination was evidenced.
- High microbiological contamination of supermarket trolleys was evidenced.

Disclosure statement

No potential conflict of interest was reported by the authors.

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