CONTAMINATION OF THE HANDLES AND BASES OF SHOPPING CARTS BY PATHOGENIC AND MULTI-DRUG RESISTANT BACTERIA

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Abstract

Background and Aims: Shopping carts (SCs) are considered as highly contaminated public surfaces, and may play a role in transmission of some harbor heterotrophic bacteria to human being. Therefore, this study aimed to examine the hygienic conditions and presence of heterotrophic bacteria on the surface of the handles and bases of SCs taken from shopping stores in Al-Mafraq city, Jordan. **Methods:** Five different SCs were selected randomly from four shopping stores (designated as A, B, C and D) during May through June, 2011. Two dry swab samples were taken from each SC, one from the handle and another one from the base. All samples were cultured on nutrient agar as none selective medium and incubated aerobically at 37 °C for 48 hours. The resulting number of colony forming units (CFUs) in each plate was converted to CFU per cm^2 surface area. The species of bacterial isolates were determined by biochemical tests and 16S rDNA sequencing.

Results: The number of heterotrophic bacteria per SC range between 6 to 133 CFU/cm^2 surface area for the cart handles and between 6 to 300 CFU/cm^2 surface area for the cart bases, indicating higher numbers of heterotrophic bacteria in the cart bases as well as more fluctuations in the number of heterotrophic bacteria at the handles and bases of SCs. These analyses also confirmed the presence of seven coliform and three noncoliform species on the tested surface of the handles and bases of the selected stores. These include *E. coli* spp., *Acinetobacter calcoaceticus, Burkholderia cepacia, Yersinia enterocolitica, Tatumeella ptyseas, Pseudomonas aeruginosa, Shigella sonnei, Bacillus cereus,* and *Bacillus pumilus*.

Conclusion: The resulting data revealed that some of these species are pathogenic as well as a multi-drug resistant. These data also indicated that poor sanitation and hygiene conditions appear to exist among the tested SCs of the selected stores. It also suggest that these SCs might act as source of transmitting of pathogens which might pose a serious health risk to shoppers. Therefore, this study pleads for a strong cleaning approaches for SCs as well as community education to promote hand hygiene, both of which are important and complementary.

Keywords: Coliforms, Fecal contamination, Shopping stores, 16S rDNA

Introduction

Shopping carts (SCs) are one of the most daily used objects by various customers in public facilities such as shopping stores (SS), malls and others. More importantly, previous studies revealed that SC handles are among the leading sources of germs and bacteria in public facilities such as shopping malls, airports and bus stations (Ghamdi et al., 2011; Reynolds et al., 2005).

According to these two studies, the total number of bacteria appeared to be higher on SC handles compared to that found in other public objects such as restaurant tabletops, escalators, chair arm rests, public restrooms and playground equipment, to name a few. In addition, it was also reported that some of these bacterial species can survive for up to several days on nonporous surfaces and are infectious at very low doses. Thus, SCs can expose shoppers, particularly children, who often ride in the carts to some harmful bacteria. In a recent report, it was mentioned that SCs can harbor several types of heterotrophic bacteria including coliforms and others (Gerba and Mawell, 2012).

Coliforms are a group of taxonomically unrelated bacteria characterized by being facultatively anaerobic Gram-negative, none sporulating, rod-shaped bacteria that ferment lactose with gas formation (Madigan et al., 2012). Examples of coliforms include *Escherichia*, *Enterobacter*, and *Klebsiella*. Coliforms can often cause several numbers of diseases or infections in healthy individuals, such as urinary tract infections, gastroenteritis, pneumonia, and much more (Madigan et al., 2012). Furthermore, due to their presence in feces of animals, coliforms are used as indicator of sanitary quality of foods and water. Thus, their presences in various objects such as SCs indicate fecal contamination and may indicate that other fecal pathogenic organisms might also be present.

In a typical day, SCs are exposed to the dripping from chicken and meat, and other wet food items as well as the hands of shoppers with poor hygiene practices, air dust and dirt from construction sites. Also, SCs

sometimes left without any type of sanitization. In addition, no sanitization standards are set for SCs by store owners and/or local health authorities. Conditions like these can lead to poor sanitation and hygiene conditions and provide an optimal environment for bacteria to grow. In addition, these conditions also increase the chance that these bacteria or microbes may transfer from SCs to employee and shoppers who usually touch or use these SCs. These bacteria may also transfer from these SCs to food items. Furthermore, based on our recent search on all available literature reviews, articles and reports, no studies to date have attempted to investigate the hygienic conditions of handles and bases of SCs from SS in Jordan. Therefore, this study was conducted to examine the hygienic conditions and the presence of heterotrophic bacteria on handles and bases of SCs from SS in Al-Mafraq city, Jordan. To accomplish this, the types and levels of heterotrophic bacteria in handles and bases of some SCs from four selected SS were determined.

Materials and Methods

Study Area and Sampling

Specimens were collected from SCs taken from four different SS from Al-Mafraq city, Jordan, during May through June, 2011. The SS were selected because of their popularity in the city and frequent visiting by customers. These stores were geographically separated as well as differently operated. These stores were assigned A, B, C, and D letters for anonymity.

Five different SCs were selected randomly from each store for sampling from the customer cart return corral to capture the recent usage ones. Two dry swab samples were taken from each SC, one from the handle and another one from the base. Factory-sealed, pre-sterilized, and disposable swabs were used in all sampling, and no preparation was needed.

A single sterile swab was wiped firmly over the entire a surface area of each shopping cart handle, from end-to-end and wiped firmly over a selected surface area of each shopping base. The surface area was recorded for each sampling area. The swab was then return back into the corresponding labeled bag. All swab samples were immediately transported to laboratory at ambient temperature and processed for cultivation of bacteria.

Bacterial growth and counting

Samples were cultured within one hour of collection by spreading the swab bacterial load on nutrient agar plate surfaces. All plates were incubated at 37 °C for 48 hours. The resulting colony forming units (CFUs) were counted for each plate and the number of colonies in each plate was then converted to CFU per cm^2.

Isolation of pure colonies

The grown bacterial colonies were morphologically compared based on size, color, margins, and elevation. Morphologically different colonies were considered as different colonies and then transferred to new nutrient agar plates to get pure cultures. The pure cultures were stored in 30% glycerol stock cultures and stored at -20 ^{o}C for further studies.

Biochemical identification of the isolated bacteria

Pure bacterial isolates were first Gram stained and subjected to biochemical tests. Oxidase test was used to determine if bacteria contain certain cytochrome C oxidase as described previously (Steel, 1961). The oxidase test was carried out using an aqueous solution (1%) of N, N, N', N'-tetramethyl-p-phenylenediamine. Based on the test, the qualitative RapID^{TM} One System (Remel, USA) was applied to identify the bacterial isolates to the species level with the help of ERIC^{TM} software.

Additionally, Gram negative bacteria were cultivated on MacConkey agar and Eosin methylene blue medium to differentiate coliforms from non-coliforms. Both media are known to be selective for Gram negative bacteria, and differentiate between bacteria based on lactose fermentation, which is a major characteristic of coliforms.

Molecular identification of pure isolates by 16S rDNA sequencing

Because we were unable to identify four isolates by the aforementioned methods, the 16S rDNA sequencing approach was used to identify these four unknown strains.

Briefly, DNA was purified from each isolate for sequencing using the Gentra pure kit (Qiagen, Valencia, CA, USA). The purification of DNA was carried out according to the manufacturer's instruction. The purified DNA was dissolved in 10 mM Tris-buffer and kept at the refrigerator at -20 ^{o}C until use. Pure DNA samples from these four isolates were sent out for sequencing.

The 16S rDNA sequencing was carried out as previously described (Jacob and Irshaid, 2012). The 16S universal bacterial primers 518F (5'-CCA GCA GCC GCG GTA ATA CG-3') and 800R (5'-TAC CAG GGT ATC TAA TCC-3') were used for amplifying the 976 bp region of 16S rDNA sequence. The DNA sequencing was done by Macrogen sequencing service facility (Macrogen Inc, Seoul, South Korea).

The resulting 16S rDNA sequences of these four unknown isolates were evaluated by comparison with the nucleotide sequence data obtained from GenBank database. The search was performed by National Center for Biotechnology Information's (NCBI) Web BLAST Service (http://ncbi.nlm.nih.gov).

Results

In this study, we intended to investigate the hygienic conditions of SCs by estimating the levels of bacterial contamination and presence of specific coliforms and other heterotrophic bacteria on the handles and bases of SCs. A total of 40 swab specimens were collected from SCs taken from four different SS (designated as A, B, C and D) located at Al-Mafraq city, Jordan.

Results of the average number of heterotrophic bacteria (CFU/cm^2 surface area) on the bases and handles of SCs taken from four different stores are presented in Table 1. The number of heterotrophic bacteria per SC range between 6 to 133 CFU/cm^2 surface area for cart handles and between 6 to 300 CFU/ cm^2 surface area for cart bases**.** The average number of heterotrophic bacteria on cart handles was almost the same for store-A, -B, -D, except for store-C, which showed slightly low number. By contrast, the average number of heterotrophic bacteria on cart bases of SCs taken from shopping store-A was higher than those taken from the cart bases of the other three stores (B, C and D). In addition, the cart bases taken from store-B contained the lowest average number of heterotrophic bacteria. The data also revealed that the average number of heterotrophic bacteria on cart bases was higher (78 ± 80) than that on cart handles (47 ± 36) for all selected stores except store-B, where cart bases and handles had almost similar levels of heterotrophic bacteria (Table 1).

Table 1. The average numbers of heterotrophic bacteria on the bases and handles of shopping carts taken from four selected shopping stores of the studied area.

Shopping store*	**Number of heterotrophic bacteria (CFU/cm^2)**	
	Handle	**Base**
A	**47 ± 25**	**108 ± 104**
B	**50 ± 23**	**37 ± 3**
C	**35 ± 35**	**85 ± 121**
D	**56 ± 59**	**81 ± 17**
Total	**47 ± 36**	**78 ± 80**

*Stores assigned letter (A, B, C, and D) for anonymity; CFU= colony forming units; Values are the mean value ± standard deviation of five shopping carts.

Nine pure different bacterial isolates, designated I-1, I-2, I-3, I-4, I-5, I-6, I-7, I-8 and I-9, were obtained from these SCs and selected for further identification using biochemical tests. By using biochemical tests, five isolates out of nine were identified. These isolates are I-1, I-2, I-3, I-4 and I-5, and identified as *Yersinia enterocolitica* (*Y. enterocolitica*), *Shigella sonnei* (*S. sonnei*), *Acinetobacter calcoaceticus (A. calcoaceticus), Burkholderia cepacia (B. cepacia*), and *Tatumeella ptyseas* (*T. ptyseas*), respectively. The biochemical properties of these bacterial isolates are presented in Table 2.

Morphological and conventional biochemical tests failed to identify the remaining four isolates (I-6, I-7, I-8 and I-9). Therefore, these four isolates were subjected to further identification using the common 16S rDNA sequence analysis. The resulted sequences were further analyzed and searched at the public databases for closest relatives at the NCBI BLAST website (http://blast.ncbi.nlm.nih.gov /blast.cgi). The partial sequences of the 16S rDNA of the four isolates (I-6, I-7, I-8 and I-9) are shown in figures 1, 2, 3, and 4, respectively. The percentage of identity of these four isolates and their closest relatives based on the 16S ribosomal target sequence are shown in Table 3. The BLAST search results showed that these four isolates match up to four different bacterial species. Based on this analysis, these four unknown isolates I-6, I-7, I-8 and I-9 have 97% identity or more to *Bacillus cereus* (*B. cereus*), *Bacillus pumilus* (*B. pumilus*), *Bacillus thuringiensis* (*B. thuringiensis*) and *Pseudomonas aeruginosa* (*P. aeruginosa*), respectively.

Table 2. The results of the qualitative biochemical tests for the five selected isolates present in shopping cart specimens taken from four selected shopping stores of the studied area.

Abbreviations of chemical tests, URE: hydrolysis of urea, ADH, hydrolysis of arginine, ODS: hydrolysis of ornithine, LDC: hydrolysis of

Test	**Isolate code** **I-1**	**I-2**	**I-3**	**I-4**	**I-5**
URE	-	-	-	-	-
ADH	+	-	-	-	-
ODS	+	-	-	-	-
LDS	-	-	-	-	-
TET	-	-	-	-	-
LIP	+	-	+	+	-
KSF	-	-	-	-	-
SBL	+	+	-	-	-
GUR	-	-	-	-	-
ONPG	-	+	-	-	-
BGLU	-	-	-	-	-
BXYL	-	-	-	-	-
NAG	+	-	-	-	+
MAL	-	-	-	-	-
PRO	-	+	-	-	-
GGT	-	-	-	+	-
PYR	+	-	-	-	-
ADON	-	-	-	-	-
IND	-	-	-	-	-
ID	**YE**	**SS**	***AC***	***BC***	***TP***

lysine, TET: utilization of aliphatic thiol, LIP: hydrolysis of the fatty acid ester, KSF: utilization of sugar aldehyde, SBL: utilization sorbitol, GUR: hydrolysis of ρ-Nitrophenyl-β,D-glucoronide, ONPG: hydrolysis of ρ-Nitrophenyl-β,D-galactoside, BGLU: hydrolysis of ρ-Nitrophenyl-β,D-glucoside, BXYL: ρ-Nitrophenyl-β,D-xyloside, NAG: ρ-Nitrophenyl-nacetyl-β,D-glucoseaminide, MAL: utilization of malonate, PRO: hydrolysis of proline-β-naphthylamide, GGT: hydrolysis of γ-glutamyl-β-naphthylamide, PYR: hydrolysis of pyrrolidonyl-β-naphthylamide, ADON: utilization of adonitol and IND: utilization of tryptophane. Identification was carried out by ERIC software. ID**:** Identification; YE: *Yersinia enterocolitica*, SS: *Shigella sonnei*; AC: *Acinetobacter calcoaceticus*; *BC: Brkholderia cepacia*; TL: *Tatumeella ptyseas*.

Table 3. The closest relatives of bacterial species present in shopping cart specimens taken from four selected shopping stores of the studied area using 16s rDNA sequence analysis.

Isolate code	**Identification Identity**	**% Identity**
I-6	*Bacillus pumilus*	99%
I-7	*Bacillus cereus*	99%
I-8	*Bacillus thuringiensis*	97%
I-9	*Pseudomonas aeruginosa*	99%

The occurrence of coliform and noncoliform bacteria on the handles and bases of the tested SCs were also investigated in this study (Table 4). The coliform bacteria were examined by their biochemical properties during growth with MacConkey agar and Eosin methylene blue medium. Seven different coliforms were identified. These include *Escherichia coli* (*E. coli*), *A. calcoaceticus, B. cepacia, P. aeruginosa, T. ptyseas, S. sonnei* and *Y. enterocolitica*.

Table 4. The occurrence of the coliform and noncoliform bacteria in shopping cart specimens taken from four selected shopping. stores of the studied area

Shopping Store	**Type of Isolate Noncoliform**	**Coliform**
A	*Bacillus cereus*	*Acinetobacter calcoaceticus* *Burkholderia cepacia* *Yersinia enterocolitica* *Tatumeella ptyseas* *Escherichia coli*
B	*Bacillus pumilus*	*Shigella sonnei*, *Escherichia coli*
C	*Bacillus thuringiensis*	*Escherichia coli*
D		*Pseudomonas aeruginosa* *Escherichia coli*

E. coli was isolated and identified from all examined stores by the distinctive metallic green sheen on the Eosin methylene blue medium and

red colonies on MacConkey agar. *A. calcoaceticus*, *B. cepacia*, *T. ptyseas* and *Y. enterocolitica* were all found in swab specimens taken from SCs of store-A. *P. aeruginosa* and *S. sonnei* were only found in swab specimens taken from SCs of store-B and D, respectively. Furthermore, three noncoliform bacilli species, *B. cereus*, *B. pumilus* and *B. thuringiensis* were isolated and identified from SCs taken from stores A, B and C, respectively.

TGGCAGAACGTATCGGCATTATTGGGCGTAAGGGCTCGCAGGCGGTTTCTTAGGTCTG
ATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGAAACTTGAGTGC
AGAAGAGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAA
CACCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTGACGCTGAGGAGCGAAAGCGTGG
GGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTG
TTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAG
TACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAG
CATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACA
ACCCTAGAGATAGGGCTTTCCCTTCGGGGACAGAGTGACAGGTGGTGCATGGTTGTCG
TCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTA
GTTGCCAGCATTTAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGG
TGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATG
GACAGAACAAAGGGCTGCGAGACCGCAAGGTTTAGCCAATCCCATAAATCTGTTCTCA
GTTCGGATCGCAGTCTGCAACTCGACTGCGTGAAGCTGGAATCGCTAGTAATCGCGGA
TCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACG
AGAGTTTGCAACACCCGAAGTCGGTGAGGTAACCTTTATGGAGCCAGCCGCCGAAGTG
GGGCAGATGATTGGGGTGAAGTCGTACAGGGGAAACCGTAAA

Figure 1. Partial 16S rDNA sequence (972 nucleotides) of the bacterial strain I-6, identified as *Bacillus pumilus*.

TCTCTTCCTAATCTTCGCGCTCAGTGTCAGTTACAGACCAGAAAGTCGCCTTCGCCACTG
GTGTTCCTCCATATCTCTACGCATTTCACCGCTACACATGGAATTCCACTTTCCTCTTCTG
CACTCAAGTCTCCCAGTTTCCAATGACCCTCCACGGTTGAGCCGTGGGCTTTCACATCAG
ACTTAAGAAACCACCTGCGCGCGCTTTACGCCCAATAATTCCGGATAACGCTTGCCACCT
ACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGGTACCGTCAA
GGTGCCAGCTTATTCAACTAGCACTTGTTCTTCCCTAACAACAGAGTTTTACGACCCGAA
AGCCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATTCCC
TACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCT
CTCAGGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCG
ACGCGGGTCCATCCATAAGTGACAGCCGAAGCCGCCTTTCAATTTCGAACCATGCGGTTC
AAAATGTTATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTATGGGCAGGTT
ACCCACGTGTTACTCACCCGTCCGCCGCTAACTTCATAAGAGCAAGCTCTTAATCCATTC
GCTCGACTTGCATGTATTAGGCACGCCGCCAGCGTTCATCCTGAGCCAGTTCAAAACTCT
GAATAATCGGTGATCCCCATCGGGCTGCGCGGGGGGGCCGCCTCCCCCCTTCTCCCACAT
ACATCACACATAAACTGCGTACATGGAGTTACTGCGTACATTCTCCTATAATTATAAGA
GGGGGGAGAAGGGTGAGCATAA

Figure 2. Partial 16S rDNA sequence (922 nucleotides) of the bacterial strain I-7, identified as Bacillus *cereus*.

GGGGGGTAACGGTTACGCATATTGGGCGTAAGGGCTCGCAGGCGGTTTCTTAAGTCTGAT
GTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGAAACTTGAGTGCAGA
AGAGGAGAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCA
GTGGCGAAGGCGACTTTCTGGTCTGTAACTGACACTGAGGAGCGAAAGCGTGGGGAGCA
AACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGG
GTTTCCGCCCTTTAGTGCTGAAGTTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCG
CAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTT
AATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAACCCTAGAGAT
AGGGCTTCTCCTTCGGGAACAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCG
TGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCATCATTAA
GTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAA
ATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACAGAACAAAGAGCT
GCAAGACCGCGAGGTGTAGCTAATCTCATAAATCTGTTCTCAGTTCGGATTGCAGGCTGC
AACTCGACTGCATGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATA
CGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTC
GGTGAGGTAACCTTTTATGGAGCCAGCCGCCTAAGGTGGGGCAGATGATTGGGGTG
AAGTCGTACAGGGGTTAACCCGTAA

Figure 3. Partial 16S rDNA sequence (975 nucleotides) of the bacterial strain I-8, identified as *Bacillus thuringiensis*

GGGGGGTTCCGTAATCGGGATTACTGGGCGTAAGCGCGCGTAGGTGGTTCAGCAAGTTGGAT
GTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCAAAACTACTGAGCTAGAGTACGGTAG
AGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGG
CGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGG
ATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTAGCCGTTGGGATCCTTGAGAT
CTTAGTGGCGCAGCTAACGCGATAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAA
CTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAAC
GCGAAGAACCTTACCTGGCCTTGACATGCTGAGAACTTTCCAGAGATGGATTGGTGCCTTCG
GGAACTCAGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAG
TCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAGCACCTCGGGGGGCACTCTAAGGAGA
CTGCCGGTGACAAACCGGATGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGC
CAGGGCTACACACGTGCTACAATGGTCGGTACAAAGGTTGCCAAGCCGCGAGGTGGAGCTA
ATCCCATAAAACCGATCGTAGTCCGGATCGCAGTCTGCAACTCGACTGCGTGAAGTCGGAAT
CGCTAGTAATCGTGAATCAGAATGTCACGGTGAATACGTTCCCGGGCCTTGTACACACCGCC
CGTCACACCATGGGAGTGGGTTGCTCCAGAAGTAGCTAGTCTAACCGCAAGGGGGACGGTT
ACCACGGAGTGATTCATGACTGGGGTG AAGTCGTACAGGGGTAAACCGTAAA

Figure 4. Partial 16S rDNA sequence (974 nucleotides) of the bacterial strain I-9, identified as *Pseudomonas aeruginosa*

Discussion

According to data obtained from this current study, the presences of heterotrophic bacteria on the handles and bases of SCs were confirmed in all four tested stores. Thus, these findings suggest that these SCs were considered as contaminated objects. These data also showed that there were variations in kinds of heterotrophic bacteria on the surface area of SCs among the selected stores. In addition, there were fluctuations in the numbers of heterotrophic bacteria at the surface of both cart bases and cart handles of

the tested SCs. These variations and fluctuations are more likely due to the degrees of cleanliness of these SCs. It may also due to types of food items that are placed in these tested SCs. These include fresh vegetables, fish, fruits and raw chicken and meat, or frozen food items. These food items and their leftovers seem to provide enough moisture and nutritional sources to support microbial growth in surfaces of the handles and bases of these SCs. Also, these variations may be due to the variations in the number of shoppers with poor hygiene practices that used these SCs and transfer these bacteria to the examined SCs.

Furthermore, it was found that the cart bases contain higher number of bacteria compared to the cart handles. This finding suggests that heterotrophic bacteria may have a better survival rate in cart base surfaces than in the cart handle surfaces. This observed variation could be attributed to the fact that the base surfaces are more likely to be exposed to contamination or less likely to be cleaned or dusted as compared to handle surfaces. As mentioned above, food items are usully placed on cart bases and their left over provide a suitable conditions for heterotrophic bacteria growth. This is in consistent with previous studies which showed that bacterial communities can be transported by dust and the total bacterial cell concentrations tend to increase in presence of both dust and moisture and nutritional sources from leftovers of the food items (Gerba and Mawell, 2012; Hara and Zhang, 2012). Therefore, clean hard surfaces are expected to harbor fewer bacteria than dirty ones.

Due to little data available about the bacterial contamination of SCs, it is difficult to establish a standard for the allowed bacterial level in SCs. In a recent report coming from United States, the numbers of the heterotrophic bacteria and coliforms among SCs were estimated (Gerba and Mawell, 2012). According to this study, the number of heterotrophic bacteria per SC ranged between 110 to 11,000,000 CFU, whereas coliform numbers ranged from less than 3 to more than 7,259 CFU per SC (668 cm^2).

In this current study, conventional and biochemical methods as well as molecular 16S rDNA sequencing revealed the presence of at least seven coliforms (*A. calcoaceticus, E. coli, B. cepacia, P. aeruginosa, T. ptyseas, S. sonnei* and *Y. enterocolitica*) and three heterotrophic bacterial species (*B. cereus, B. pumilus* and *B. thuringiensis*) in the handles and bases of SCs. This is in agrrement with previous reports which revealed that SCs are among the leading sources of germs and bacteria in various public facilities, including shopping malls, airports and bus stations (Reynolds et al., 2005; Ghamdi et al., 2011). In addition, the distribution of these heterotrophic species throughout the tested SCs of these stores were not uniform or similar, rather each species appeared to be associated with certain SCs of a specific store. For instance, *E. coli* appears to be the most common bacterial species

in the handles and bases of SCs for all examined stores. Other mentioned bacterial species such as *B. cereus*, *B. pumilus*, *B. thuringiensis*, *P. aeruginosa* and *S. sonnei* only appears once on some SCs taken from a specific store. Thus, these findings revealed that these SCs play key roles in the harboring of some pathogenic and potential pathogenic heterotrophic bacteria.

Taken together, the presence of these coliforms and noncoliform bacteria among these SCs is an indicator of poor sanitation and hygiene conditions. Thus, if these SCs are left without sanitization or disinfection, they may play a key role in transmission of these bacteria from person to person, and can cause serious diseases to human being. Interestingly, according to World Health Organization report, diseases caused by poor sanitation and bad hygiene rank among the leading causes of hospitalization and ill-health in population worldwide (World Health Organization, 1999).

E. coli, *T. ptyseos* and *Y. enterocolitica* belong to the well-known family of *Enterobacteriaceae* and are part of the fecal coliforms that can cause illnesses in humans (Janda and Abbott, 2006; Forsythe, 2010). As a human pathogen, *Y. enterocolitica* is most frequently associated with acute diarrhea, terminal ileitis, mesenteric lymphadenitis, and pseudoappendicitis (Bercovier et al., 1980). *T. ptyseos*, a fermentative Gram-negative rod bacterium, has rarely been reported as a cause of human infections; however, there is very little information about it in the medical literature (Costa et al., 2008). The presence of these fecal coliforms on any object is an indicator of fecal contamination from human or cattle as well as indicator of inadequate of sanitary quality of foods and water. Based on these data, it is possible to suggest that the source of these species might be the hands of individuals who harbor these species or contaminated vegetable or food items that are placed on these shopping carts.

P. aeruginosa is a well-known multidrug resistance pathogen and very common form of soil bacteria. It is associated with nosocomial infections. The mechanisms of virulence include secreted toxins and the ability to form biofilms (Shanthi and Sekar, 2005; Ruxana et al., 2005; Driscoll et al., 2007). This organism may reach objects such as SCs via direct contact with infected persons. It is also possible that this bacteria may come from air dust and/or soil dirt from construction sites near these selectedstores.

S. sonnei and *B. cepacia* are all Gram negative bacteria. *S. sonnei* is known to cause shigellosis and can be transmitted from person to person by a fecal-oral route (World Health Organization, 1999). *B. cepacia* most often causes severe pulmonary infection and cepacia syndrome and might cause damage to the lung and eventually lead to death in immunocompromised individual (Mahenthiralingam et al., 2005). This species could be present on

various hard object surfaces including sinks, counter tops. Thus, it is possible to suggest that the potential sources of *S.* and *B. cepacia* are the hands or the sputum of customers or employee who might be infected with these pathogens. The presence of these pathogens in these SCs *may pose a* dangerous threat to human being.

A. calcoaceticus, formerly known as *Achromobacter anitratus*, was also detected in this study. This species is present as normal flora of the skin and throat of human beings along with other saprophytes (Pal and Kale, 1981). However, it was proved that this organism is an opportunistic pathogen and it was connected to several diseases like urinary tract infections. It is likely that the potential sources of *A. calcoaceticus* are the hands and sputum of customers or employee.

Based on the 16S rDNA sequence, three *Bacillus* species (*B. pumilus*, *B. cereus*, and *B. thuringiensis*) were identified. These *Bacillus* species are Gram positive, rod-shaped, aerobic and spore-forming bacteria (Madigan et al., 2012; Tena et al., 2007; Vilain et al., 2006). *Bacillus* species can withstand a range of variable environmental conditions due to their structure and ability to form spores (Helgason et al., 2000; Wijnands et al., 2006). This feature give them advantages to spread in the various environments and ultimately in SCs. They also appear in soil, contaminated water, chronically infected wound and on dead plant tissue.

B. pumilus may cause infection to humans. It was also indicated that this species could cause cutaneous infections and the lesions appeared to have a morphology similar to that of cutaneous anthrax lesions (Tena et al., 2007). *B. thuringiensis* has limited capability to cause disease in humans. However, this species produces a toxin that inhibits the growth of a wide number of insect larvae and hence it has been used as a biological pesticide (Helgason et al., 2000; Granum, and Lund, 1997). *B. cereus*, on the other hand, is a soil bacterium and an opportunistic pathogen that was connected to food poisoning (Helgason et al., 2000; Vilain et al., 2006). Despite the fact that these three *Bacillus* species are placed in the same genus, they were found in three different stores. This finding may suggest that these *bacillus* species could have originated from same or similar sources of contamination. However, we cannot also exclude the possibility that these three species may have come from different sources of contaminations.

It is an important to mention that during our examinations of the areas near these stores, there is evidence that some new buildings that are under construction are very close to the selected stores. In addition, our visual examinations of these selected SCs indicated that these SCs were not being cleaned effectively. These observations suggest that the close proximity of studied stores to construction sites could be easily lead to facilitate the contamination of SCs by air dust or soil dust coming from these

sites. These conditions will most likely promote the growth of these species. This may explain our finding of these soil bacteria on the surface of both the handles and bases of the tested SCs.

Moreover, there are other important reasons for presence of these pathogenic and potential pathogenic bacteria as well as for such observed fluctuations and variations in the numbers and types of these heterotrophic bacteria. These include a warm temperature, humidity, crowded living conditions, and poor sanitary conditions (Hahn et al., 1999; Jugnia et al., 2001; Harsha et al., 2007; Gerba and Mawell, 2012, Akoachere et al., 2013). These samples were collected during May and June of 2011. In terms of the weather in Jordan, May and June have nice warm weather in general. Temperatures at these two months are around 17-31°C (www.jdtours.com/en/jordan/weather). Most bacrteia are mesophiles, they can grow well at room temperature or temperature between 20 to 30 °C. In addition, most heterotrophic bacteria isolated from soil often grow best at 20 to 30 °C. Therefore, the conditions mentioned above creat and provide a good environment for these types of bacteria to grow and thirive.

These pathogenic bacteria may also come from both customers and employees with poor hygiene conditions as well from those who are infected with these pathogenic bacteria. These conditions will ultimately increase the chance that these species may grow and thrive on surfaces of these carts, which in turn, transmit these bacteria to unsuspecting employees and coustemers. It is also worth to mention that the hygiene and sanitary practices in these stores were similar, hence there was not much difference in number of bacteria between these selected stores. Exposure to these pathogenic bacteria together with poor hand hygiene might lead to spread some of the infectious diseases. Some of infectious disease can be devastating, and sometimes fatal, particularly, those associated with multidrug-resistant gram-negative bacteria. Therefore, to protect employees and customers from these pathogens and prevent these pathogens to contaminate products and cause illness, the owners of these stores or employers must improved sanitary conditions in their facilties and encourage good hygiene behaviours. To accomplish these gaols, employers must implement a daily schedule for cleaning and sanitizing the surfaces of those SCs in their stores. In addition, they must assure that there are compliance and self adherence with hand hygiene practice among their employees. These improvements would help to reduce the presence of infectious pathogens associated with a disease.

Conclusion

Variations in the number and types of bacterial species between the SCs taken from these selected stores were observed. In addition, at least

seven species of coliforms and three species of heterotrophic bacteria were isolated and identified in the surface of the handles and bases of SCs. Some of these species are pathogenic or potential pathogenic as well as a multi-drug resistant. These findings confirm the notion that the SCs might act as source of transmitting of pathogens from person to person. Thus, it is possible to suggest that these pathogenic species constitute a serious health threat to local communities, governments and public at large. Globally, these pathogens will continue to pose a direct risk to the human well-being and animal health.

The results of this study indicated that poor sanitation and hygiene conditions exist among these tested SCs of the four selected stores. These findings from our study are also recognizing the need to take urgent measures to improve the sanitary conditions of these SCs. Therefore, removing soil, dust and dirt from SCs are necessary steps on the reduction of surface contamination by microbes. We also recommend that these cleaning approaches must be undertaken in parallel with community education for hygienic standards. These include, but are not limited to promote the development of successful sanitation programs or techniques and increased sanitation investment as well as promoting hand hygiene and putting these cleaning approaches into action. These measurements could reduce the risk of serious infectious diseases that might be caused by these pathogenic and multi-drug resistant bacteria existing in the surface of the handles and bases of SCs.

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