CHAPTER ONE

1. INTRODUCTION AND OBJECTIVES

1. 1. Introduction

Retail stores that use wheeled shopping carts for the collection of goods from a store, such as a supermarket, usually are pushed from behind. In some instances, a small child, is placed in the collapsible seat adjacent the handle of the shopping cart so that the shopper can have his or her hands free for shopping while being positioned close to the child.Under the above noted circumstances, the hands of the shopper and possibly the hands of the child of the shopper come in contact with the shopping cart handle. Contaminants present on the cart handles are spread by this contact(Al-Ghamdi*et al.*,2011).

The spread of microbesstarts when hands touch the cart handle and the hands are subsequently placed on other surfaces that are touched by others, Shopper will handle or touch trays containing raw meat and/or raw poultry while making the decision. Usually traces of blood and other liquid matter exuded from then raw contents is present on the exterior of the package, but these contaminants on the outside of the trays are difficult to detect and are contacted by the unwary shopper(Georgianna,2007).

The evidence now seemed to infer that not only are the surfaces of grocery shopping cart handles "dirty," they can also be sources of disease (Burgess *et al.*,2005;Harrison*etal.*,2001).

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Bacterial contamination is most spread in supermarkets in the shopping carts handles. All of shopping carts are places that are most touched by the bare hands of people who are in various hygienic conditions. In fact 80% of infections are spread by shopping carts, through hand contact with hands or other objects. Some microbes are infectious at very low doses and can survive for hours to weeks on nonporous surfaces and hand pieces (Reynolds*et al.*, 2005).

Enterococci have been found to survive in dry conditions and on various fabrics utilized in the health care environment. Infectious doses of pathogens may be transferred to the mouth after handling an everyday contaminated household object (Rusin*et al.*,2002).

Recentlystudies have demonstrated that health care workers' hands were contaminated with various types of microorganisms on shopping carts handles. The percentage of contamination is varying in their hygienic status. Moreover, the items that shoppers hold in their hands vary in the degree of cleanliness. The in-shop handling of different items is another fact significantly. People who push the shopping carts vary in their hygienic status. The in-shop handling of different items is another fact significantly of different items is another factor that determines hand hygiene. Fluctuation between items such as fresh vegetables, fruits and then fresh dripping chicken, fish or frozen items would subject the hands to dampness and make them patient for picking up microbes. Those samples obtained from elevator buttons of shopping malls and of residential areas revealed nearly the same percent of contamination as those of other objects flora including potentially pathogenic bacteria such as *Staph. aureus* and Gram negative bacilli can be obtained from various sources in the

environment.Some of which have been mentioned earlier such as shopping cart handles and supermarkets. Other sources could be contaminated surfaces, shaking hands with carriers of diseases or with patients (Ulger *et al.*, 2009).

Shopping carts and handheld shopping baskets in supermarkets or retail grocery stores are subjected to accidental bacterial contamination through contacts with a variety of food such as raw meats, fish and chicken, However the level of bacterial contamination on shopping carts or shopping baskets is not a subject of public health concern because of the limited availability of information (Jones *et al.*, 2006; Fullerton *et al.*, 2007).

Clinical investigations indicate that infection risks depend on numbers of organisms transferred and the immune status of the person in which Potentially pathogenic bacteria isolated from shopping cart include Gram –negative bacilli (Scott and Bloomfield, 2008).

Bacteria that can cause severe gastroenteritis can be readily transferred from hands to almost any frequently used surface like shopping cart and it's the role in the transmission of disease and remains as controversial subject (Roxburgh, 2005).

Recent studies have shown that children are at increased risk of both *Salmonella* and *Campylobacter*infections if they ride in a shopping cart carrying meat products (Jones *et al.*, 2006; Fullerton *et al.*, 2007; Patrick *et al.*, 2010).

This suggests that exposure of children to enteric bacterial pathogens in shopping carts occurs on a regular basis (Mizumachi *et al.*, 2010).

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Reynolds*et al.*, (2005) reported frequent exposure to pathogenic *Staph.aureus*on shopping cart handles and suggested that this was a hidden reservoir of this organism and the need for shopping baskets sanitation. Contamination of shopping carts may occur from direct handling of raw food products and contamination of the cart from previous users, total bacterial levels in shopping cart are far greater than found in public restrooms and other public places and objects that are commonly touched in the environment such shopping malls.

1.2. Rationale

Most people do not realize that microbes are found on many common surfaces such as supermarkets shopping trolleys and baskets that are most touched by the bare hands of people who are in various hygienic condition (Al-Ghamidi *et al.*, 2011). Recently the number of supermarkets was increased rapidly in Khartoum and other fudavese towns. This increasing may be due to change in nutritional habits and style of life. Poor handling and use of unsafe raw materials such as fruits and meats within trolley and basket can result in bacterial contamination. To our knowledge, there are no published studies, to date, in Sudan that have specifically investigated the type and levels of bacterial contamination on supermarket shopping trolleysand baskets.Therefore this study was conducted to assesscontamination of supermarket shopping baskets and trolleysby pathogenic or potentially pathogenic Gram-negative bacteria in Khartoum Locality.

1.3. Objectives

1.3.1. General objective

To assess Gram-negative bacterial contamination on supermarket shopping baskets and trolleys in Khartoum Locality.

1.3. 2. Specificobjectives

- 1) To collect swab samples from supermarkets' shopping trolleys and baskets.
- 2) To determine bacterial load on shopping trolleys and baskets.
- 3) To identify of Gram negative bacteria on shopping trolleys and baskets.

CHAPTER TWO

2.LITERATURE REVIEW

Shopping carts are considered of the most importance tools for shopping in supermarkets. It helps you buy all household, including your personal supplies, and transfer those purchases throughout the store and car parking space, but there is problem of it contamination with different microbes. Al-Ghamdi*et al.*, (2011)investigated the status of bacterial contamination of shopping carts handles. A total of 400 samples were collectedfrom different places in supermarkets in the city of Jeddah, Saudi Arabia. 95.5% of the total samples collected were contaminated with mixed bacterial growth. Coagulase-negative staphylococci dominated the isolates. The second most common bacterial growth in all specimens was Gram-positive bacilli. Potential pathogens isolated from all specimens were: *Staph. aureus*, *Pseudomonas* spp. ,and Gram- negative bacilli.

Shopping carts and handheld shopping baskets in supermarkets are subjected to accidental bacterial contamination through contacts with a variety of food. Studies investigated the prevalence of *Staphylococcus aureus* on the handles of handheld shopping baskets in four supermarkets distantly located in Osaka district, Japan, *Staphylococcus aureus* was isolated as one of the classical aetiological agents of food poisoning (Kai and Igarashi, 2001).

It is well known that cross-contamination can occur when handling raw food and that proper hand-washing amongst the general public such as shopping carts can be lacking(Hopkins, 2009; Kyriacou*et al.*,2009).

Fluctuation between items such as fresh vegetables, fruits and then fresh dripping chicken, fish or frozen items would subject the hands to dampness and make them picking up microbes. Samples obtained from elevator buttons of shopping malls and of residential areas revealed nearly the same percent of contamination as those of other objects formerly mentioned transient flora including potentially pathogenic bacteria such as *Staph. aureus* and Gram-negative bacilli(Ulger *et al.*, 2009).

The hands of health care workers may become persistently colonized with such bacteria(Gram-negative bacteria and Gram-positive bacteria) and spread it to others outside the healthcare premises through hand shaking or through touching various objects such as shopping carts (Al-Ghamdi*et al.*,2011).

Placement of children in grocery shopping carts has recently been implicated as a source of infection with *Salmonella* and *Campylobacter* infections in young children. This confirmed with the study that was conducted to assess the occurrence of total numbers of bacteria, coliform bacteria and *Escherichia coli* on grocery shopping cart handles and seats. A total of 85 shopping carts in parking lots of grocery stores were tested in five major methropoltian areas across the United States. The total number of heterotrophic bacteria averaged 117,000 per sampled area. Coliforms were detected on 72% of the carts, and *Escherichia coli* identified on 50% of the carts tested. Shopping carts appear to be one of the most bacterially contaminated objects that the general public may come into contact on a regular basis in public facilities. The exceptionally high level of coliform bacteria suggests that fecal material may be involved in cart contamination. The results

of this study reaffirm the need for improved sanitation of shopping trolleys/baskets to reduce exposure and potential transmission of microbial infections among shoppers (Gerbaand Maxuell,2010).

Cross-contamination of trolleys and baskets occurs when disease-causing microorganisms are transferred from one food to another. For example, raw meat products are often contaminated with foodborne bacteria such as *Salmonella* and *Campylobacter*(Doyle, and Beuchat, 2011).

Although cooking of foods usually destroy these bacteria, the organisms may be transferred to other foods that are sometimes consumed uncooked, or may contaminate the hands of consumers and be directly transferred to the mouth, resulting in infection. Transfer may occur by surfaces such as cutting boards and kitchen counter tops as well as by the hands (Rusin*et al.*, 2002).

Other studies showed that shopping cart handles have recently been targeted by the popular press as a highly contaminated public surface with fecal-bacterial and food-borne pathogen. Contaminations on local shopping cart handles are detected utilizing a combination of molecular methods and traditional cultivation techniques. The study investigated the total aerobic bacterial populations present, as well as identified the presence of *Escherichiacoli* spp., *Salmonella* spp., and Shiga toxin producing *Escherichia coli* (STEC) on shopping cart handles from retail grocery stores in the Sacramento Region. This method used PCR to identify generic *Escherichia coli* as an indicator of fecal contamination. Out of 600 samples, one sample (0.17%) tested positive for

Staphylococcus toxygenic*Escherichia coli*and one sample tested positive for *Salmonella* spp. (0.17%). For the fecal contamination indicator test, 582 were found to be positive for *Escherichiacoli* spp. (97%). The total number of aerobic bacteria found on the cart handles varied from 0 to over 53,000 colony forming units (Morris, 2003).

The common occurrence of coliform and *Escherichia coli* bacteria on shopping carts indicate that the consumer is exposed to fecal bacteria on a regular basis when using grocery shopping carts, Total bacterial levels are far greater than found in public restrooms and other public places and objects that are commonly touched in these environments (i.e. airports, bus stations, public bathroom, shopping malls, etc(Reynolds *et al.*, 2005).

Gerbaand Maxuell(2010) found that bacteria on these shopping carts ranged from 5 to 41.5 sq. cm. with the higher average found in public restrooms. Coliforms and *Escherichia coli* also appear to be in greater numbers on shopping cart handles than other common surfaces consumers may come into contact. In testing of diaper changing tables, chair arm rests, playground equipment, automated machine button, restaurant tabletops, escalators, restaurant condiments coliforms were only detected on 7% (16/200 samples) (Reynolds *et al.*, 2005) vs. 72% on shopping carts in study reported by Gerba and Maxuell (2010).

Coliform bacteria usually originate from feces and are associated with poor sanitary conditions. Coliform bacteria and *Escherichia coli* detected on the carts may originated from contact with raw foods, birds (while sitting in the parking lots between use) or other

animal feces, contact with fecally contaminated hands or other body parts (hands). The high numbers of bacteria and coliform bacteria indicate extreme unsanitary conditions of the carts compared to other public places and surfaces that the general public comes into contact. This increases the risk of coming into contact with a disease causing organism, Results of several epidemiological studies have shown that a risk of infection from common enteric bacteria is related to placement of small children in shopping carts (Jones *et al.*, 2006; Fullerton *et al.*, 2007; Patrick *et al.*, 2010).

Other study was assessed the cross-contamination of food products by reusable bags used to carry groceries. The reusable bags were collected at random from consumers as they entered grocery stores in California and Arizona. In interviews, it was found that reusable bags are seldom if ever washed and often used for multiple purposes. Large numbers of bacteria were found in almost all bags and coliformbacteria in half. *Escherichiacoli* were identified in 8% of the bags, as well as a wide range of enteric bacteria, including several opportunistic pathogens. When meat juices were added to bags and stored in the trunks of cars for two hours, the number of bacteria increased 10-fold, indicating the potential for bacteria in bags by > 99.9%. These results indicate that reusable bags, if not properly washed on a regular basis, can play a role in the cross-contamination of foods (Williams*etal.*, 2011).

Shopping carts handheld shopping in supermarkets are subject to accidental bacterial contamination through contacts with a variety of food. The study done in Japan

investigated the prevalence of Staphyloccucs areus(Staph.areus) on the handles of handheld shopping baskets in four supermarkets distantly located in Osaka district, Japan. Fifty two strains of Staph. aureus were isolated from 760 baskets handles. Among these, six strains were positive for staphylococcal enterotoxin B (SEB) production. Representing 12% of total, this SEB producer ratio is considerably higher than among Staph. aureus isolated from nasal swabs of supermarket workers (2%) and from independently collected clinical specimens (4%)(Wiley, 2011). This SEBproducing *Staph. aureus* strains from the basket handles are clonal and belong to ST12. Coagulase typing showed that they are in group VII, which is the most common cause of food poisoning in Japan. Biofilm assays indicated that SEB gene (seb)-positive strains including this clone produced a significantly higher amount of biofilm than seb-negative strain (Souazet al., 2009).

The frequent isolation of *Staph. aureus* on shopping baskets handles raises the possibility that they could be a hidden reservoir for *Staph. aureus* with a potential to cause (*Staph. areus*) food poisoning and draws attention to the importance of shopping baskets sanitation (Wiley, 2011).

The study done by Ashgar and Said (2012) showed the hygiene of environmental surfaces from shopping, play role in spreading fecal and total coliform bacteria as well as pathogenic bacteria. The study addresses the contaminated common sites by pathogenic or potentially pathogenic The study done by said showed hygiene of environmental bacteria, the total samples 422 were negative bacterial count (71%) and 226 (29%) were positive.

All collected samples (100%) of glass windows in the fish markets were bacterial counted; most dominated was *Bacillus* spp. (n = 97) and the highest population of species was *Enterococcus faecalis*(n = 40) and *Escherichiacoli* (n = 16).

CHAPTER THREE

3. MATERIALS AND METHODS

3.1. Study design

3.1.1. Study type

This is a descriptive cross- sectional study.

3.1.2. Study area

The study was carried out in selected supermarkets in Khartoum Locality. Laboratory investigation was done in the Research Laboratory, College of Medical Laboratory Science, Sudan University of Science and Technology (SUST).

3.1.3. Study duration

The study was conducted during the period from March to September2014.

3.2. Collection of samples

Samples were collected from supermarket trolleys and baskets representing different area of Khartoum Locality. Sterile cotton swabs were moistened with sterile normal saline and then trolleys and baskets handles were sampled. Each swab was put in a plain container containing 2ml sterile normal saline then transferred to the Research Lab immediately (Morris, 2003).

Aserial dilution were done by taking 1 ml homogenized sample added to sterile test tube containing 9 ml sterile normal saline and mixed properly by vortex machine (Robert and Greenwood, 2003).

3.3. Culture of samples

Under a septic condition near Bunsen burner, the specimens were mixed by vortex, mixture then diluted 10 fold with sterile normal saline, then the last three dilutioninoculatedinnutrientagar in threePetridishesand mixed, then they allowed to dry and incubatedovernight at 37°C.

3.4. Bacterial load

Serial dilutions of each sample was made using sterile normal saline as diluents, one ml of the dilution was placed into each of three sterile Petri dishes. About 15 ml of molten clear nutrient agar was added to each plate with temperature 45^{0} C. Each plate was mixed well by moving it five times in a vertical, clockwise, horizontal and anticlockwise direction. All plates were incubated at 37^{0} C. For 24 hour (Collee*et al.*, 1996).

3.5. Identification of the bacterial isolates

3.5.1. Gram stain

Smear was prepared and allowed to dry by air, then fixed by passing over the flame three times, the fixed smear covered with crystal violet stain for 30-60 second. The stain washed rapidly by clear water tiped off all water and cover with lugol's iodine for 30-60

second, the iodine was washed with clean water, decolorized rapidly (few second) with acetone alcohol, and washed immediately with clean water. Then the smear was covered with neutral red stain for 2 minute, the stain was washed with clean water, the back slide was wiped cleaned cotton and was placed in draining rack for the smear to air dry.

The smear examined microscopically, first with 40 x objective to check the staining and to see distribution material and then with oil immersion to look for bacteria (Cheesbrough, 2000).

3.5.2.Biochemical tests

3.5.2.1.Oxidase test

The oxidase test is used to determine the bacteria that produce certain cytochrome oxidase enzyme, which catalyze the transport of electron between the electron donors in the bacteria and a redox dye (tetramethyl .P.phenylenediamine) the dye is reduced to deep purple color.

By using disc impregnated with a reagent such as tetramethyl .p.phenylenediaminedihydrochloride (TMPD),which is a redox indicator.Oxydase disc were placed on sterile petridishes, and colonies to be tested were picked up with a wood and smear made, deep purple color within5-10 seconds indicate positive result(Collee*et al.*, 1996).

3.5.2.2.Urease test

The test is used to determine the ability of the organism to produce the enzyme urease, which hydrolyze urea. When the strain is urease producing, the enzyme will break down the urea (by hydrolysis)to give ammonia and carbon dioxide, with the release of ammonia. The organism is cultured in a medium which contain urea and the indicator phenol red. The medium becomes alkaline as shown by change in colour of the indicator to pink –red.A slope of urea agar medium incubated with test organism and examined after 24 hour of incubation. Change of the color to red indicates positive reaction(Cheesbrough,2000).

3.5.2.3. Indole test

This test demonstrates the ability of certain bacteria to decompose the amino acid tryptophan to indole, which accumulates in the media. Indole is then tested for by colorimetric reaction with P-dimethyle-aminobenzaldhyde. Tryptophan broth was then inoculated with test organism and incubated for 24 hours at 37°C. 5 ml of kovacs reagent added and shacked gently, Ared color in the alcohol layer indicate positive reaction (Collee*etal.*, 1996).

3.5.2.4. Motility test

This test is used to test movement of bacteria by show turbidity after inoculums. The test was done by using semisolid agar. Whichby adding of 0.2-0.5 % of agar into nutrient broth. In a semisolid media motile bacteria (swarmed) and gave diffuse spreading growth

that is easily recognized by naked eye, thus may be detected more easily than microscopically (hanging drop)method (Collee*etal.*, 1996).

3.5.2.5. Citrate utilization test

This test is one of several technique used occasionally to assist in based on the ability of an organism to use citrate as its only source of carbon, and tested for the ability of an organism to utilize citrate as sole carbon and energy source for growth and ammonium as sole source of nitrogen, Simmons citrate inoculated by test organism and incubated at 37°C for 24 hours. A green color indicates positive result (Collee*etal.*, 1996).

3.5.2.6. Fermentation of sugar, H₂S and gas production

The fermentation of sugar a, production of hydrogen sulphide and gas production was carried out by using kligler iron agar. Kiligler iron agar (KIA Oxoid Company) tubes Inoculated with test organism by using sterile straight wire, bystabbing button firstly then the slop streaked. Then tube Closed with sterile cotton swab and incubated at 35- 37° Covernight. Yellow slope indicates lactose fermentation, yellow butt indicates glucose fermentation, red color indicates no fermentation and blacking in the media indicates H₂S production (Cheesbrough, 2000).

CHAPTER FOUR

4. RESULTS

Two hundred swabs were collected from supermarket shopping trolleys and baskets. The swabs were examined to assess Gram-negative bacterial contamination.

The swabs were taken from 10 supermarkets in Khartoum Locality. These supermarkets and number of samples from each areshown in table (1). All samples were cultured on standard bacteriological media to determine Bacterial load. Of the two hundred swabs examined, 181(90%) demonstrated bacterial growth. The rest 19 (10%) failed to show any bacterial growth (Table 2). The bacterial load was calculated in term of colony forming unit (CFU).The result revealed that the mean bacterial load was 8.557×10^6 CFU/ml. The mean bacterial load of each supermarket was as follows (CFU/ml):

Supermarket (1) 247×10^4 , Supermarket (2) 153×10^4 , Supermarket (3) 3×10^4 , Supermarket (4) 3×10^4 , Supermarket (5) 52×10^4 , Supermarket (6) 47×10^4 , Supermarket (7) 35×10^4 , Supermarket (8) 48.2×10^4 and two branches of Supermarket (9) 239.5×10^4 (Table 3).

Qualitative analysis of bacterial isolates revealed39 (19%)Gram-negative bacilli. Of these *Escherichia coli* 6 (15%), *KlebsiellaPneumoniae* 2(5%), *Pseudomonas aeruginosa* 7(18%) *Shigella* spp.3 (8%) and *Salmonella* spp. 21(54%) (Table 4).

Table 1.Distribution of samples according to their sources

Location	NO	%
Supermarket (1)	20	10
Supermarket (2)	20	10
Supermarket (3)	10	5
Supermarket (4)	10	5
Supermarket (5)	20	10
Supermarket (6)	5	2.5
Supermarket (7)	15	7.5
Supermarket (8)	15	7
Supermarket (9)	85	43
Total	200	100

Table 2. Bacterial growth after primary cultivation of samples

Specimen	No	%
Positive growth	181	90
Negative growth	19	10
Total	200	100

Table 3.Mean bacterial load according to supermarket

Location	Mean bacterial load (CFU/ml)
Supermarket (1)	247×10^4
Supermarket (2)	153×10^4
Supermarket (3)	3×10 ⁴
Supermarket (4)	3×10^4
Supermarket (5)	5210 ⁴
Supermarket (6)	47×10^4
Supermarket (7)	35×10^4
Supermarket (8)	48.2×10^4
Supermarket (9)	239.5×10 ⁴
Total	855.7×10 ⁴

Table 4. Identification of Gram-negative bacteria

Gram-negative bacteria	No	%
Escherichia coli	6	15
Klebsiellapneumoniae	2	5
Pseudomonusaeruginosa	7	18
Shigellaspp.	3	8
Salmonellaspp	21	54
Total	39	100

CHAPTER FIVE

DISCUSSION

5.1. DISCUSSION

Swab samples from 10 supermarkets were collected and examined for bacterial contamination. The bacterial contamination was detected in 181(90%). This result is almost similar to that reported by Al-Gamdi*et al.* (2011) who found that bacterial contamination on supermarket was 93%. The result confirmed the fact that the shopping carts appear to be one of the most bacterially contaminated objects (Gerba and Maxuell, 2010).

Overall assessment of shopping baskets and trolleys samples analyzed bacteriologically indicated high bacterial load 85.57×10^5 CFU/ml. This result of current study disagrees with finding reported by Morris, (2003) who found that bacterial load was 53×10^4 CFU/ml. The probable reason of discrepancy may be due to geographical variation and seasonal variation.

The present study, successfully found that 39(19%) were Gram-negative bacteriathis result is more than that reported by Al-Ghamdi*et al.* (2011) who found Gram-negative bacteria was 12(11%). Both studies reported potentially pathogenic bacteria on trolleys and baskets.

The results of this study revealed a wide variation between supermarket trolleys and baskets within the same store. This variability suggests that contamination levels may also be depend on the previous user/s of the cart, or the times in which the cart is used.

A number of factors may influence the relative contamination levels between cart users. Among these, the use of available sanitation options, personal hygiene, children present, type and number of items placed within the cart, and time spanbetweenuse of carts (Morris, 2003).

Isolation of *Salmonella* spp., *Escherichia coli*and*Klebsiella pneumonia* in this study is in agreement with finding reported by Morris, (2003) and Gerbaand Maxuell, (2010).

The presence of these bacteria demonstrates shopping baskets and trolleysdo get contaminated by pathogenic organisms and a risk from food borne pathogens does exist (Williams, 2011).

The present study found that *Salmonella* spp. constituted 21(54%) of Gram-negative bacteria recovered. This finding disagrees with Gerbaand Maxuell, (2010) who failed to isolate any salmonella spp. The presence of large number of *Salmonella* spp. generally indicated poor hygiene of users and/or lack of sanitation of these carts often use.

Presence of *Escherichia coli*isolates 6(15%) in this study is less than that 582 (97%) reported by Morris (2003).*Escherichia coli* appear to be in a greater numbers on shopping carts handles than other common surfaces consumers may come into contact. This fact was reported by Reynolds *et al.*, (2005) when testing of changing tables, chair arm rests,

playground equipment, automated machine button, restaurant tabletops, escalatorsand restaurant condiments. However, another study conducted by Gerbaand Maxuell, (2010) reported that 72% of *Escherichia coli*appear on shopping carts.

The present study also identified members of enterobacteriaceae other than *Salmonella* spp. and *Escherichia coli* such as*Pseudomonas aeuroginosa*7(18%) and *Shigella*spp.*3*(8%).However, isolation of these organisms in shopping carts has not beenreportedbefore. The probable reason of discrepancy may be due to geographical variation and seasonal variation.

.5.2 CONCLUSION

The level of bacterial contamination was very highin supermarket trolleys and baskets (90%). The presence of *Escherichia coli* suggests that fecal contamination.

5.3. RECOMENDATIONS

1. Sanitation of shopping trolleys/baskets may play role in reducingbacterial contamination using disinfectant as contained in a wipe.

2. Two solutions to reduce exposure to consumers are to provide the consumer with a disinfectant as contained in a wipe and the use of disposable barriers with antimicrobial adhesive, encourages supermarket to offer complementary sanitary wipes, Disposable plastic barriers are design to fit over the hand contact area, such as the handle of the trolleys and baskets, and then be discarded in a recycle bin after use or by the next user. These barriers contain antimicrobial adhesive.

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Appendices

a. Acetone alcohol

Equal volume of ethanol (90%) and acetone solution are mixing.

b. Crystal violet stain

Crystal violet 20 g in absolute ethanol 195 ml, 9g of ammonium oxalate in200mlofdistiled water mixed together with stain solution, the volume completed with distilled water until one liter.

c. Gram iodine

Piotassiumiodide20 g and iodine 10g dissolved in one liter distilled water .

d. Kovac reagent

P-dimethylaminobenzaldhyde 10gm in amyl or isoamyl alcohol150ml, the volume completedto200 by adding concentrated HCL.

e. Normal saline(90% physiological saline)

9g salt in 70ml distilled water as the salt dissolved completely the volume complete to 100ml.

f. Safranine solution

25g of safranine powderin 100 ml of 95% ethanolealcohol.

Appendices 2

a. Indole medium

Formula of tryptophan broth

Peptone or pancreatic digest f casein		2gm
sodium chloride		0.5gm
Distilled water	100ml	

Preparation

Dissolve the ingredient in water by heating.Autoclave for 15 minutes t 121 -3°

Dispense in a test tube

b. Kililer iron agar

Formula /Liter

Enzymatic digest of casein	10gm
Enzymatic digest of animal tissue	10gm
Lactose	10gm
Dextrose	1gm
Ferric Ammonium Citrate	0.5gm
Sodium Chloride	5m

SodiumThiosulfate	0.5gm
Phenol Red	0.025gm
Agar	15gm

Preparation

52 grams of the medium in one liter of distilled water. Sterilization at 121°C (15Ibs. pressure) for 15 minutes in autoclave. Cool and pour the media in a slanted position so to obtain butts of 1.5-2cm. Depth .

c. Nutrient agar

15 gm nutrient agar powder in one litter of distilled water, ten sterilized by autoclaving at 121°Cfor15minutes. Cooled to about 50°Cand poured into sterile petri dishes in 15ml amount. The poured media left to solidify at room temperature.

d. Urea agar

Enzyme digest of gelatin	1gm
Dextrose	1gm
Sodium chloride	5gm
Monopotassium	2gm
Urea	20gm
Phenol red	0.012gm

Preparation

29g of the urea base in 100ml ofpurified water until dissolved completely. Autoclave at 121°C for 15minutes . Cool sterilized aar to 45-50°C and aseptically add the sterile urea agar base. Then mixed thoroughly and dispense into sterile tubes in a slanted position.

e. Simmons citrate agar

Formula per litter

Magnesium sulphate	0.2gm
Ammonium dihydrogen phosphate	1gm
Dipotassium phosphate	1gm
Sodium citrate	2gm
Bromothymol blue	0.08gm
Agar	15gm

Preparation

Suspend 24.28 grams in 1000ml distilled water. Heat, to boiling to dissolve the medium completely. Mix well and distribute in tubes or flasks. Sterilize by autoclaving at 15 Ibs pressure (120°C) for 15 minutes. Final PH is 6.8.a