CHAPTER ONE
INTRODUCTION AND OBJECTIVES

1.1. Introduction

One of the first shopping carts was introduced on June 4, 1937, the invention of Sylvan Goldman, owner of the Piggly Wiggly, supermarket chain in Landrige, another shopping-cart innovator was Oral Watson, who invented the swinging rear door to allow for "nesting" in 1946 (Terry, 1978).

Most people think the food shops as nice clean place, but in fact as study in United State showed that there may be unwelcome microorganisms on the handle of the trolley (Gerba and Maxwell, 2012).

Recent investigations have not only identified shopping cart handles as one of the most biologically contaminated public surfaces, but also have implicated riding in shopping carts as a risk factor for food-borne pathogen infection in infants (Fullerton *et al*., 2007).

A logical source of contamination of grocery store shopping carts would be from the consumer's hands. The presence of enteric bacteria on hands and surfaces has been well studied within high-exposure environments such as medical and childcare communities (Manzur *et al*., 2008).

Another source for bacteria on shopping cart handles could potentially be from the raw foods and packaging that are handled in the grocery store and placed within the cart (Blanco *et al*., 2003).

Mizumachi *et al*., (2010) reported frequent exposure to pathogenic *Staphylococcus aureus* on shopping cart handles and suggested that this was a hidden reservoir of this organism and the need for shopping basket sanitation. Contamination of shopping carts may occur from direct handling of raw food products or contamination of the cart from previous users (Mizumachi *et al*., 2010).

A new study into the hygiene of supermarkets has found that shopping carts are dirtier than the store's bathrooms. Gerba of the University of Arizona conducted research on the handles of 85 carts in four American States. He reported bacteria from human waste on the handles of 72 percent of them. Nobody seems to routinely clean and disinfect shopping carts (Gerba and Maxwell, 2012).

1.2. Rationale

Despite the great expansion in trade and the spread of malls and supermarkets in the Sudan in the recent years, but there is no reported on the possibility of the presence of bacterial contamination on the trolley and basket which may expose customers for many diseases. Therefore, the aim of this study was to assess whether the shopping basket and trolley use in the supermarket are contaminated with Gram-positive bacteria or not.

1.3. Objectives

1.3.1. General objective

To assess Gram-positive bacterial contamination on shopping basket and trolley in Khartoum Locality during the period from April to August 2014 using bacteriological methods.

1.3.2. Specific objectives

a) To determine bacterial load on shopping basket and trolley.

b) To isolate and identify bacteria found on shopping basket and trolley.

c) To determine percentage of isolated G-positive bacteria.

CHAPTER TWO

2. LITERATURE REVIEW

At supermarkets and grocery stores, someone are exposed to many surfaces, such as refrigerator door handles and shopping carts that are touched by hundreds of people each day. With every touch, these surfaces become contaminated with more and more potentially harmful bacteria. However, there are a few measures supermarkets and grocery stores can take to help minimize the spread of these microbes. Clean hands are one of the most effective ways to prevent illness and the spread of germs (Duberg, 2011).

In 2012, shopping carts were identified as a source of germs and became a major public health concern. This was primarily because of the media spotlight on a Japanese research study indicating a large amount of bacteria was found on shopping carts (Gerba and Maxwell, 2012). This was confirmed in 2011 when the University of Arizona released a study called, "Research Report on Shopping Cart Bacterial Contamination " (Sobotka, 2011).

Another study conducted in 2012, which proves shopping cart handles to be "hidden reservoirs" for pathogenic *S. aureus*. The team of this study collected samples by swabbing the seat and handle from a total of 85 shopping carts. The samples were then packed in ice and delivered overnight to the University of Arizona for processing. The total estimated surface area sampled among the 85 shopping carts was 668 square

centimeters. The outcome of the study proved that 72-percent of the 85 shopping carts sampled contained by pathogenic bacteria (Gerba and Maxwell, 2012).

A total of 85 shopping carts in parking lots of grocery stores were tested in the United States. The total number of heterotrophic bacteria averaged 117,000 per sampled area. Shopping carts appear to be one of the most bacterially contaminated objects that the public may come into contact on a regular basis in public facilities (Gerba and Maxwell, 2012).

Most people do not realize that microbes were found on many common objects outdoors, in their offices, and even in their homes. Such objects include; playground equipments, ATM keyboards, kitchen sinks, office desks, computer keyboards, escalator handrails, elevator buttons and with the spread of supermarkets and hypermarkets the shopping carts handles. All of the latter objects are places that are most touched by the bare hands of people who are in various hygienic conditions. People believe that microbes are only present in research labs or in hospitals and clinics and thus they have a misleading feeling of security in other places. Lack of knowledge about where microbes prowl could be the cause of health problems. In fact, 80% of infections are spread through hand contact with hands or other objects (Reynolds *et al.*, 2005).

Reynolds *et al.*, (2005) used an invisible fluorescent tracer for artificial contamination of public surfaces, they found that contamination from outside surfaces was transferred to 86% of exposed individual's hands and 82% tracked the tracer to their home or personal belongings hours later. The viability of Gram-positive and some Gram- negative

organisms under various environmental conditions have been described (Noskin *et al*., 1995). Some microbes are infectious at very low doses and can survive for hours to weeks on nonporous surfaces, such as countertops, telephone hand pieces and shopping carts handles (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).

Epidemiological study by Jones *et al.*, (2006) and Fullerton *et al*., (2007) identified riding in shopping carts near meat or poultry products as an associated risk factor in infant infections.

The combined results of these studies suggest that shopping carts may in-fact, play a role in the transmission of pathogenic bacteria (Galan and Curtiss, 1991).

Reynolds, (2005) conducted a research to determine how certain bacteria find their way into people's homes. She visited six Tucson, Arizona supermarkets. At the laboratory, Reynolds made a starting discovery: one in five carts tested positive for bodily fluid (blood, mucus, saliva or urine) that could transmit infectious germs. Reynolds found *S. aureus, S. pneumoniae, E. coli* and even hepatitis.

Study to investigating the status of bacterial contamination of four daily used objects, computer keyboards, computer mice, elevator buttons and shopping carts handles. 400 samples were collected from four different objects; 100 from each. Samples were collected from different places (offices, internet cafes, homes, buildings and

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: *S. aureus*, *Pseudomonas* spp. and Gram negative bacilli. The presence of pathogenic and commensal bacteria on the four objects indicates that they might act as environmental vehicles for the transmission of potentially pathogenic bacteria (Al-Ghamdi *et al*., 2011).

Scott and Bloomfield (2008) suggested that, where contaminated surfaces come into even relatively brief contact with the fingers or an inanimate surface, a significant number of organisms can be transferred which could be recoverable onto an agar surface. In this study, Gram-positive bacteria were more frequently isolated from all surfaces compared to Gram-negative. This could be in part because survival of Gram-positive species on laminate surfaces is greater than that of Gram-negative organisms (Scott and Bloomfield, 2008). However, Gram-positive and Gram-negative bacteria have been shown to have similar transfer rates from laminate surfaces to fingertips (Scott and Bloomfield, 2008).
Zuke in 2012 take swabs from 120 trolley handles across the big five revealed a cocktail of bacterial species would eliminate. *S. aureus* was present on all 120 trolleys, while 68-hosted *Campylobacter*, 36 were home to *E. coli,* and revealed trace of *Listeria* (Zuke, 2012).

CHAPTER THREE

3. MATERIALS & METHODS

3.1. Study design

This is a descriptive cross sectional study conducted to assess the bacterial contamination on supermarkets trolley and baskets on Khartoum Locality.

3.1.1. Study area

Study was conducted in supermarkets located in different localities in Khartoum Locality. The laboratory investigation was carried out in the Research Laboratory, Sudan University of Science and Technology

3.1.2. Study duration

Study was carried out during period from April to August 2014.

3.1.3. Sample size

A total of 100 basket and trolley were tested for contamination with Gram-positive bacteria from customer's hands.

3.2. Collection of samples

The trolleys and baskets were swabbed with a sterile cotton wool swabs moistened in sterile norml saline. The swab was rotated on all handle parts of each trolley and basket.

The cottony part of the swab was placed in 2ml of sterile normal saline. Laboratory analysis was conducted as quick as possible.

3.3. Bacterial load

3.3.1. Preparation of serial dilutions

Serial dilutions of each sample were made using sterile normal saline as diluents.Ten fold serial dilutions were prepared by transferring 1ml of the stock suspension to 9ml of diluents in test tube to obtain dilution of 1/10. The step was repeated up to dilution of $1/10^6$ in the last tube (6).

3.3.2. Pour Plate method

Pour plate method was carried out. The number of living bacteria in the dilution was counted. 1ml of the dilution was mixed with nutrient agar medium in Petri dish. After incubation, the number of colonies was counted in CFU/ml. only plates between 30-300 colonies were selected.

a. 1ml of the dilution was placed into each of three sterile Petri dishes.
b. About 15ml of molten nutrient agar was added to each plate with temperature 45°C.
c. Each plate was mixed well by moving it five times in a clockwise and anticlockwise direction.
d. All Plates were incubated at 37°C for 24 hr (Collee *et al*., 1996).

3.3.3. Calculation

All of the Petri plates containing between 30 and 300 colonies were selected. Plates with more than 300 colonies were excluded and are designated too many to count (TMTC). Plates with fewer than 30 colonies were designated too few to count (TFTC). The colonies were calculated on each plate. The number of bacteria calculated as colony forming unit (CFU) per milliliter multiplied the number of colonies by the dilution factor dividing by the amount of specimen added to liquefied agar (Collee *et al*., 1996).

3.4. Identification of bacteria

3.4.1. Gram's stain

Smear was done by emulsified the colonies picked from an overnight growth in sterile normal saline. The smear was left to air dry, then fixed by flame. The smear was covered with crystal violate stain for 30-60 seconds, rapidly washed off the stain with clean water. Then covered with Lugol’s iodine for 30-60 seconds, rapidly washed with clean water. Decolorized rapidly with acetone alcohol, washed immediately with clean water. Finally the smear was covered with safranine stain for 2 minutes and then washed with clean water. The back of the slide was wipped clean and left to air dry. The smear was examined microscopically by oil immersion objective (Cheesbrough, 2000).

3.4.2. Biochemical testes

1. Catalase test

Two to three ml of 3% hydrogen peroxide was poured into a test tube. Using a sterile wooden stick, a portion of a good growth of the tested organism was transferred, and then immersed in the hydrogen peroxide solution. Immediate bubbling is positive result (Cheesbrough, 2000).

2. Coagulase test

Coagulase is an enzyme that causes plasma to clot. The test used to differentiate *S. aureus*, which produce coagulase enzyme from other staphylococci. 0.5ml of diluted plasma was placed in small test tube. 5 drops of bacterial suspension was added and then mixed gently, incubated at 37°C for up to 4 hours, and then examined for clot formation (Cheesbrough, 2000).

3. DNase test

This test was used to identify *S. aureus*, which produce deoxyribonuclease enzyme. DNase hydrolyses deoxyribonucleic acid (DNA). The test organism was cultured on a medium, which contain DNA. After overnight incubation at 37°C, the colonies were tested for DNase production by flooding plate with a weak hydrochloric acid solution (1mole). DNase producing colonies were surrounded by clear area due to DNA hydrolysis (Cheesbrough, 2000).

4. Mannitol fermentation

Test organism was inoculated into Mannitol Salt Agar, incubated at 37 °C and then examined after 24 hours for mannitol fermentation. It was indicated by formation of yellow colonies (Collee *et al.*, 1996).

5. Sugar fermentation test

Fermentation is a type of microbial metabolism in which bacteria breakdown organic compound to get energy. It results in various end products like acid, gases, both acid and gas or other end products. Bacteria under test were inoculated in different sugars broth media (glucose, mannose, maltose, sucrose, xylose, and trehalose) which contain nutrient red as indicator. Tubes of various sugar media were selected, labeled and inoculated with test organism aseptically. Tubes were incubated at 37°C for 24-48 hours with non-inoculated tubes as control. Color changes were observed (Pommerville, 2005).

3.5. Statistical analysis

After completion of data collection, each measurement of different variables was recorded according to the workflow. Data entry and analysis was done using SPSS version 16.

CHAPTER FOUR

4. RESULTS

One hundred trolley and basket were assessed for bacterial contamination, as well as the types of contaminating bacteria.

Fifteen samples were collected from Alwaha supermarket, thirty-seven from Aswaquna I and forty-eight from Aswaquna II (Table 1).

Eighty one (81%) out of 100 trolley and basket were contaminated with bacteria while the remaining 19(19%) showed no bacterial growth. The mean of bacterial load in these supermarkets was 191.9 $x10^4$ CFU/cart. The mean bacterial load in different supermarkets was found as follows; in Alwaha supermarket was 96.4 $x10^4$ CFU/cart, in Aswaquna (I) 318.4 $x10^4$ CFU/cart and 161 $x10^4$ CFU/cart in Aswaquna (II) supermarket. (Table 2)

Sixty-six (66%) isolates were identified as Gram-positive bacteria. From sixty six Gram-positive bacteria, most of them were *Bacillus* spp. 22(33.3%) followed by *S. hominis* 12 (18.2%), *S. aureus* 11 (16.7%), *S. warneri* 10 (15.2%), *S. haemolyticus* 6 (9.1%), *S. xylosus* 3 (4.5%), *S. saprophyticus* 1 (1.5%) and *S. epidermidis*, 1 (1.5%) (Table 3).

Table 1. Bacterial growth and percentage among the supermarkets basket and trolley

Supermarket	samples		%
	Total	Growth	
Alwaha	15	7	46.7
Aswaquna(1)	37	33	89.2
Aswaquna(2)	48	41	85.4
Total	100	81	-

Table 2. Mean of contamination in the supermarkets basket and trolley

Supermarket	Mean of contamination	
	CFU/ml	CFU/cart
Alwaha	48.2 $x10^4$	96.4 $x10^4$
Aswaquna 1	159.2 $x10^4$	318.4 $x10^4$
Aswaquna 2	80.5 $x10^4$	161 $x10^4$

The mean contamination in the three supermarkets was 95.5 $x10^4$ CFU/ml and 191.9 $x10^4$CFU/cart.

Table 3. Frequency and percentages Gram-positive bacteria isolated from supermarkets basket and trolley

Bacteria	Frequency	%
Bacillus spp.	22	33.3
S. hominis	12	18.2
S. aureus	11	16.7
S. werneri	10	15.2
S. haemolyticus	6	9.1
S. xylosus	3	4.5
S. epidermidis	1	1.5
S. saprophiticus	1	1.5
Total	66	100%

5. DISCUSSION

5.1. Discussion

A shopping trolley and basket were used every day by the shoppers for transport their purchase. However, studies implicated shopping trolley and basket as one of most biological contaminated public surfaces. There is no published Sudanese study examining shopping trolley and basket contamination. The occurrence of bacteria on the handles of shopping trolley and basket was assessed in three different supermarkets located in Khartoum Locality. The results of this study demonstrated that the majority of swabbed trolley and basket were contaminated with bacteria, most of which are common skin flora coagulase-negative staphylococci.

The rate of shopping trolley and basket contamination was 81%. This contaminated trolley and basket might act as environmental vehicles for transmission of microbial infection among shoppers. Almost similar results were obtained in Jeddah by Al-Ghamdi *et al.*, (2011) and in USA by Gerba and Maxwell, (2012). Who reported the contamination rate as 95.5% and 72% respectively.

In this study, the average of bacterial load was 191.9x10^4CFU/cart. This finding is higher than that reported by Gerba and Maxwell, (2012) 34.3x10^4CFU/cart.

Gram-positive bacteria isolated during this study represent (66%). This could be in part because survival of Gram-positive species on laminate surfaces is greater than that of Gram-negative organism (Scott and Bloomfield, 2008). Resident floras, which are attached to deeper layers of skin, are more resistant to removal by routine washing.

Coagulas-negative staphylococci and Gram-positive diphtheroids are members of this group (Boyce and Pittet, 2002).

The Gram-positive isolates recovered in this study were coagulase-negative staphylococci (51%) followed by *Bacillus* species (33.3%) and *Staphylococcus aureus* (16.7%).

Presence of coagulase-negative staphylococci is lower than that reported by AL-Ghamdi *et al*., (2011) (87%). Moreover, presence of *Staphylococcus aureus* is in agreement with AL-Ghamdi *et al*., (2011) (14%) but lower than that reported by Zuke, (2012) (100%).

The high numbers of *S.aureus* indicate extreme unsanitary conditions of the carts compared to other public places and surfaces that the 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 was related to placement of small children in shopping carts (Jones *et al*., 2006; Fullterton *et al*., 2007; Patrick *et al*., 2010).

Most disinfecting wipes provided today contain quaternary ammonium based compounds which require at least 10 minutes contact time to be effective against many organisms (Block, 2001). Disposable plastic barriers are design to fit over the hand contact area, such as the handle of the cart, and then be discarded in a recycle bin after use or by the next user. These barriers contain antimicrobial adhesive on one side.

5.2. Conclusion

In conclusion, supermarkets shopping trolley and baskets appear to be one of the most bacterially contaminated objects that the public may come into contact on a regular basis in public facilities. The exceptionally high levels of *Bacillus* spp. and *S. aureus* suggest human hands contamination.

5.3. Recommendations

1. Improved sanitation or the use of antimicrobial adhesive barrier devices, which prevent cross-contamination among products and shoppers, appears justified.
2. Provide hand sanitizer gives customers the opportunity to disinfect their hands upon entry to avoid contaminating shopping trolley and basket.
3. Regular cleaning and disinfecting handle of shopping trolley and basket are highly recommended.

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APPENDIX

Equipments

Microscope	Olympus optical co.Ltd.japan
Incubator	Toree pice Nardi (CR), Italy
Auto clave	U.K
Swabs	Local products
Sterile containers	Pyrex , U.S.A
Sterile petridishes	Pyrex , U.S.A
Slides	Supert , Germany
Test tubes	Pyrex , U.S.A
Microbiological loops	Local products

Media

The following media were used:

1. Nutrient Agar

It is basic medium used for count the bacteria.

2. MacConkey Agar

It is differential medium used to distinguish between lactose fermenting from non-lactose fermenting bacteria.

3. Mannitol Salt Agar

It is used to differentiate *S. aureus* from other Staphylococci.

4. Deoxyribonuclease Media (DNase)

It is used to identify bacteria which produce DNase enzyme.

Reagents

0.9% Physiological saline.

8.6% Hydrochloric acid.

100% Absolute alcohol.

3% Hydrogen peroxide (H2O2).

Stains

The following stains were used:

Crystal violet.

Lugol's iodine.

Safranin.

Suger fermentation

Peptone water.

1g sugar (glucose, sucrose, maltose, mannose, trehalose, xylose).

Nutrient red.

Appendix 2

Count			
Sample code	CFU/ml	CFU/cart	Isolated organism
1	19 x 10^4	38 x 10^4	*S. aureus*
2	5 x 10^4	10 x 10^4	*S. xylosis*
3	84 x 10^4	168 x 10^4	*P. aeruginosa*
4	11 x 10^4	22 x 10^4	*S. warneri*
5	10 x 10^4	20 x 10^4	*S. aureus*
6	40 x 10^4	80 x 10^4	*S. aureus*
7	458 x 10^4	916 x 10^4	*S. haemolyticus*
8	16 x 10^4	32 x 10^4	*Bacillus* spp.
9	14 x 10^4	28 x 10^4	*Salmonella* spp.
10	41 x 10^4	84 x 10^4	*S. hominis*
11	15 x 10^4	30 x 10^4	*S. aureus*
12	40 x 10^4	80 x 10^4	*Bacillus* spp.
13	20 x 10^4	40 x 10^4	*S. hominis*
14	36 x 10^4	72 x 10^4	*S.aureus*
15	47 x 10^4	94 x 10^4	*S. haemolyticus*
16	63 x 10^4	126 x 10^4	*S. aureus*
17	9 x 10^4	18 x 10^4	*S. warneri*

18	-	-	No
19	166 x10^4	332 x 10^4	*S. haemolyticus*
20	53 x10^4	106 x 10^4	*Bacillus* spp.
21	391 x10^4	782 x 10^4	*Bacillus* spp.
22	209 x10^4	418 x 10^4	*S. haemolyticus*
23	294 x10^4	588 x 10^4	*E. coli*
24	-	-	No
25	-	-	No
26	234 x10^4	468 x 10^4	*S. xylosis*
27	8 x10^4	16 x 10^4	*S. warneri*
28	26 x10^4	52 x 10^4	*S. aureus*
29	11 x10^4	22 x 10^4	*E.coli*
30	15 x10^4	30 x 10^4	*Bacillus* spp.
31	32 x10^4	64 x 10^4	*S. hominis*
32	-	-	No
33	4 x10^4	8 x 10^4	*Bacillus* spp.
34	3 x10^4	6 x 10^4	*K. pneumonia*
35	30 x10^4	60 x 10^4	*S. aureus*
36	35 x10^4	70 x 10^4	*Bacillus* spp.
37	203 x10^4	406 x 10^4	*P. aeruginosa*

38	458 x10^4	916 x 10^4	*Bacillus* spp.
39	498 x10^4	996 x 10^4	*S. xylosis*
40	680 x10^4	1.360 x 10^4	*S. saprophyticus*
41	256 x10^4	512 x 10^4	*Bacillus* spp.
42	275 x10^4	550 x 10^4	*Bacillus* spp.
43	-	-	No
44	-	-	No
45	300 x10^4	600 x 10^4	*Bacillus* spp.
46	196 x10^4	392 x 10^4	*S. warneri*
47	209 x10^4	418 x 10^4	*S. warneri*
48	294 x10^4	588 x 10^4	*S. hominis*
49	166 x10^4	332 x 10^4	*S. aureus*
50	-	-	No
51	89 x10^4	178 x 10^4	*S. aureus*
52	33 x10^4	66 x 10^4	*Bacillus* spp.
53	20 x10^4	40 x 10^4	*Bacillus* spp.
54	60 x10^4	120 x 10^4	*S. epidermidis*
55	43 x10^4	86 x 10^4	*Bacillus* spp.
56	33 x10^4	66 x 10^4	*S. hominis*
57	187 x10^4	374 x 10^4	*Bacillus* spp.

58	169 x10^4	392 x 10^4	*E. coli*
59	-	-	No
60	364 x10^4	728 x 10^4	*Bacillus* spp.
61	37 x10^4	74 x 10^4	*Bacillus* spp.
62	-	-	No
63	12 x10^4	24 x 10^4	*Shegilla* spp.
64	-	-	No
65	-	-	No
66	64 x10^4	128 x 10^4	*E. coli*
67	-	-	No
68	37 x10^4	74 x 10^4	*K. pneumonia*
69	19 x10^4	38 x 10^4	*Bacillus* spp.
70	22 x10^4	44 x 10^4	*Bacillus* spp.
71	35 x10^4	70 x 10^4	*S. hominis*
72	-	-	No
73	70 x10^4	140 x 10^4	*S. warneri*
74	15 x10^4	30 x 10^4	*S. hominis*
75	20 x10^4	40 x 10^4	*S. haemolyticus*
76	5 x10^4	10 x 10^4	*Bacillus* spp.
77	-	-	No
78	23 x10^4	46 x 10^4	*Shigella* spp.

79	32 x10^4	64 x 10^4	*Bacillus* spp.
80	204 x10^4	408 x 10^4	*S. hominis*
81	22 x10^4	44 x 10^4	*S. hominis*
82	51 x10^4	102 x 10^4	*S. hominis*
83	45 x10^4	90 x 10^4	*Salmonella* spp.
84	58 x10^4	116 x 10^4	*E. coli*
85	18 x10^4	36 x 10^4	*Shigella* spp.
86	-	-	No
87	124 x10^4	248 x 10^4	*S. warneri*
88	285 x10^4	570 x 10^4	*P. aeruginosa*
89	-	-	No
90	80 x10^4	160 x 10^4	*Bacillus* spp.
91	37 x10^4	74 x 10^4	*S. hominis*
92	-	-	No
93	45 x10^4	90 x 10^4	*S. haemolyticus*
94	38 x10^4	76 x 10^4	*S. hominis*
95	55 x10^4	110 x 10^4	*S. haemolyticus*
96	58 x10^4	116 x 10^4	*S. warneri*
97	55 x10^4	110 x 10^4	*S. warneri*
98	-	-	No

99	84 x10^4	168 x 10^4	*S. warneri*
100	-	-	No