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# Survival of some Egyptian bacterial isolates in different water types

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**Abstract :** Survival of bacteria in water exerts a public health concern. The aim of this work was to evaluate the survival of some pathogenic bacteria isolated from El-Rahawy drain and maintained for 4 months at room temperature in different water sources.
The initial bacterial counts for each bacterial isolate were$10^4$ cfu/ml. Water samples were weekly collected from each tested water types for bacterial count using plate count agar poured-plate technique. Results showed that *Pseudomonas aeruginosa* and *Bacillus subtillus* were still alive in all tested water sources even after the end of the experiment (16 weeks).
In **sterilized distilled water** samples**,** complete $\log_{10}$ reductions were observed at the first and fourth weeks for *Salmonella* spp. and *E. coli*, respectively. Each of *Staphylococcus aureus*and *Streptococcus faecalis* reached complete die-off point at the third week of incubation.
Concerning **sterilized tap water** samples**,** complete $\log_{10}$ reductions were observed for *E. coli*, *Salmonella* spp., *Staphylococcus aureus* and *Streptococcus faecalis* at the $6^{th}$, $4^{th}$, $5^{th}$ and $12^{th}$ week of incubation, respectively.
In **sterilized groundwater** samples, viability of *Streptococcus faecalis* bacteria exceeded over the period of experiment with $\log_{10}$ reduction 3.5 cfu / ml, but other tested bacteria (except *Pseudomonas aeruginosa* and *Bacillus subtillus*) reached the die-off point during the experiment. Surprisingly, the log count of *Pseudomonas aeruginosa* showed increase in cell numbers from the $3^{rd}$ week until $8^{th}$ week by $\log_{10}$ counts ranging from 0.1 to 0.5 cfu/ml.
Regarding **sterilized seawater** samples**,** complete $\log_{10}$ reductions occurred for *E. coli, Salmonella* spp., *Staphylococcus aureus* and *Streptococcus faecalis* at the $7^{th}$, $5^{th}$, $13^{th}$ and $9^{th}$ week of incubation, respectively.
In conclusion, preservation of water having the possibility of bacterial contamination may exert public health hazards.
**Keywords** : Bacterial isolates, Survival, Distilled Water, Tap Water, Groundwater, Seawater.

## 1. Introduction

Human beings accustomed to use water mainly for drinking, washing and bathing as well as other different purposes. Water pollution significantly affects the general health consumers and users (WHO,[1]).

Naturally, different water types can harbor myriads of different microorganisms. However, various factors play a role in survival of microorganisms in water such as water activity, organic matter, temperatures, type of microorganisms, number of organisms as well as type of water (WHO,[2]).

Some researchers (Iacobellis&DeVay,[3] and Liao & Shollenberger,[4]) reported that bacteria including *Agrobacterium tumefaciens* and *Pseudomonas* spp. could survive in sterile distilled water for several years.

Also, Uyanik, etal[5] observed that *Salmonella flexneri* can survive in both 0.9% NaCl solution and distilled water for 87 and 83 days, respectively. But, in Northern Ireland Kerr, etal[6]observed that *E. coli* O157:H7 detectable on 42 and 14 days in sterile natural mineral water and sterile distilled deionized water at 15 to $20^0$C, respectively. As well asin Spain, Serrano, etal[7] concluded that distilled water could reduction or killing (0.99 $log_{10}$cfu/ml) of *Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa* after 24 hours than chlorinated tap water. Moreover, by the structure of the spore, Anthony, etal[8] reported that in the present harsh environmental conditions and without nutrients, bacteria spores can survive for long periods (several months or years).

On the other hand, tap water is the main source for drinking and other domestic. The safe of drinking water effected directly on human health than any other thing, and the lack of safe drinking water due to the problems especially in developing countries (Parson and Jefferson,[9]).Sakyi and Roland,[10] reported that the numbers of coliform group and heterotrophic plate count bacteria in environmental stress (chlorinated water), are decreased as well as could not survive for 2 to 3weeks. While Abd El-Salam, etal[11], found that *Pseudomonas aeruginosa* are surviving longer than one year in bottled water brands in Egypt. Also, in Cameroon, Djaouda, etal[12]concluded that, if any bacterial member of coliform or *Vibrio cholera* or *salmonella* spp. arrived to treated water (which used for drinking water), they can regrowth and survival for a long time (several weeks) depended on temperatures and water conditions as well as causing diseases for human consumer. Moreover, in Egypt El-Tokhy, etal[13] isolated *Pseudomonas putida biotype A*, *Citrobacter freundii* and *Aeromonas hydrophila* DNA Group1 from River Nile, they have ability to survive in treated water in present Fe; Mn and Al rich to 5 mg/l concentration and suggested that they may be could survival in drinking water for long time (several weeks).

In addition, in Egypt, groundwater is considered important water source which used for different purposes to a consumer. Lewis, etal[14] observed that pathogenic bacteria can survival in ground water for 100 days or more. In addition, they concluded that, bacteria can survival in groundwater is longer than in surface water, this may be absence of sunlight, lower temperatures and competition for available nutrients as well as chemical nature of the groundwater. In addition, Conboy and Goss,[15]reported that, the main source bacteria in ground water came from human activity due to contaminated it, moreover, they reported bacterial able transport through soils and able to survive in it for several weeks.

Also, in USA, John and Rose,[16] noticed that, inactivation rates were approximately 0.07-0.1 $log_{10}$/day-1 for coliform bacteria, *Enterococcus spp.*, *Salmonella* spp, coliphage and poliovirus in groundwater samples.

On the other side, seawater is considerable a different environment to variety of microorganisms. Some bacteria loving in freshwater but can survival in marine water is called halophilc bacteria like *Pseudomonas* spp. and *Vibrio* spp. The lethal to many microorganisms in this aqua medium are higher salt concentration, lower nutrients and unsuitable pH for microorganisms (Karner, etal[17]). Some studies (Carlucci & Pramer,[18]; Anderson, etal[19] and Rozen & Belkin,[20]) monitored *E. coli* isolate in seawater for 8 days at selected salinities (1, 1.5, 2.5, and 3%):they observed survival *E. coli* increasing when salinity decreasing, moreover they reported polluted materials when arrived to seawaters, enteric bacteria can able survived for long time in seawater. Moreover, Hernroth, etal[21]suggested that *Escherichia coli*, *Salmonella enterica* and *Vibrio parahaemolyticus* play role as a source of enteric infection because they able survival in marine environment for several years. In addition, Crictina and Ardelean,[22]observed that *E. coli* can survival for several weeks in seawater at different temperatures (4 and $37^o$C). Tiruvayipati and Bhassu,[23]elucidated that *Vibrio parahaemolyticus* can living and multiplication in salinity (0.8 and 3 %) water caused some diseases for marine life.

Thus, the main objective of this study highlights on survival and behavior of some Egyptian bacterial isolates in sterile different water sources.

## Material and Methods

### Water samples

Four types of water were used in this study; distilled water, tap water, groundwater and seawater. All water samples were collected under aseptic conditions in clean sterile polypropylene autoclavable containers **(APHA,[24]).**

Tap water was collected from microbiology laboratory, National Research Centre, Cairo, Egypt. Distilled water was collected from water distillation system (Aquatron, A-8000) in the laboratory. Groundwater was collected from El- Rahawy region, Giza governorate, Egypt. Also, seawater was collected from Al-Agamy beach, Alexandria governorate. All collected water types were separately distributed in flasks (capacity 5L) and then autoclaved at 121ºC for 15 min.

### Preparation of bacterial isolates

Six different bacterial (*E. coli, Salmonella* spp., *Pseudomonas aeruginosa, Bacillus subtillus Staphylococcus aureus and Streptreptococcus faecalis*) isolates were used in this work. All bacterial isolates were obtained from El-Rahawy drain, Giza, Egypt. Water samples were collected from the subsurface layer (at depth 30 cm) in fifth sterile bottled glass (1 liter capacity). One liter of drain water wascollected and 10ml from it were filtered using membrane filter technique. Hi Media (Mumbai, Maharashtra, India) were used for detection and identification of the formerly described bacterial isolates according to APHA,[24].

A loop-full from one specific colony for each bacterial isolate was transferred to 5ml tripticase soy broth tube and incubated at 37°C for 24 hours. After incubation, the tubes were centrifuged at 5000 rpm for 15 minutes.The obtained pellets were separately transferred to 5ml sterile saline water and then vortexed. The washing, centrifugation and vortexing steps were repeated three times then the bacterial solution was ready for use.

### Quantification of bacterial isolates and inoculation of different water types

Number of colony forming units in each of the obtained bacterial suspension was determined using Plate Count agar according to APHA,[24]. The sterilized different water types were separately dispensed equally in flasks and the calculated counts of bacterial isolates were separately injected in these flasks taking in consideration that the final concentration of each bacterial isolate in each flask was $10^4$cfu/ml. Each bacterial isolate was examined against all tested types of water, separately.

### Examination of survival time

All flasks were stored for 16 weeks (from August to November, 2015) at room temperature. During this period, one ml water sample was taken weekly from each flask to calculate the total viable bacterial count by using Plate Count agar plates that were incubated at 37$^o$C for 24 hours (APHA,[24]**).**

### Statistical analysis:

Statistical analysis was performed as for two factorial randomized complete blook design (Gomez and Gomez[25]).

## Results and Dissection

### Survival bacterial isolates in sterile distilled water

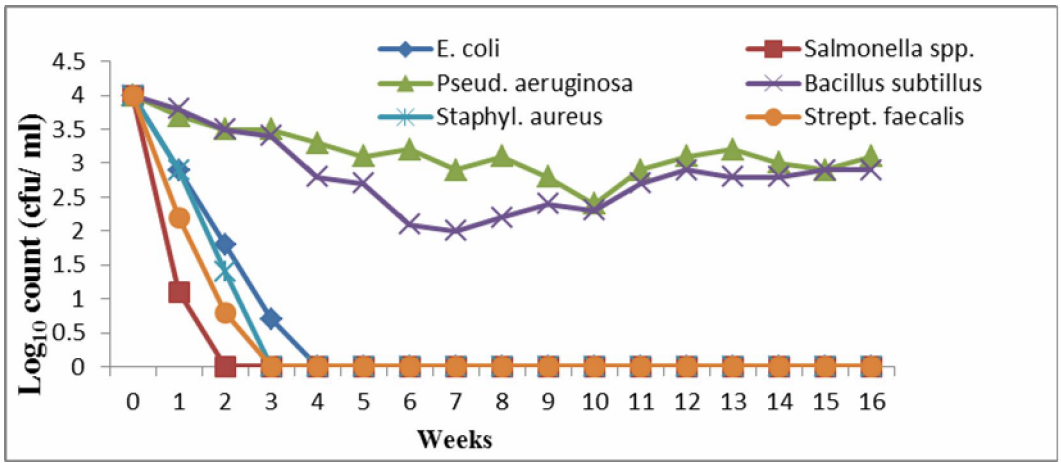
Data given in Table (1) and illustrated by Fig. 1 show$\log_{10}$reduction values (cfu/ml) of different bacterial isolates*(E. coli, Salmonella* spp.*,Pseudomonas aeruginosa*, *Bacillus subtillus, Staphylococcus aureus* and *Streptococcus faecalis*) in sterile distilled water for 16 weeks at room temperature.

**Table 1:- Reduction $log_{10}$ values (cfu/ml) of different bacterial isolates in sterile distilled water for 16 weeks at room temperature.**

| Incubation time (weeks) | Bacteria isolates | | | | | |
|---|---|---|---|---|---|---|
| | *E. coli* | *Salmonella* spp. | *Pseud. aeruginosa* | *Bacillus subtillus* | *Staphyl. aureus* | *Strept.f aecalis* |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 1.1 | 2.9 | 0.3 | 0.2 | 1.1 | 1.8 |
| 2 | 2.2 | 4 | 0.5 | 0.5 | 2.6 | 3.2 |
| 3 | 3.3 | 4 | 0.5 | 0.6 | 4 | 4 |
| 4 | 4 | 4 | 0.7 | 1.2 | 4 | 4 |
| 5 | 4 | 4 | 0.9 | 1.3 | 4 | 4 |
| 6 | 4 | 4 | 0.8 | 1.9 | 4 | 4 |
| 7 | 4 | 4 | 1.1 | 2 | 4 | 4 |
| 8 | 4 | 4 | 0.9 | 1.8 | 4 | 4 |
| 9 | 4 | 4 | 1.2 | 1.6 | 4 | 4 |
| 10 | 4 | 4 | 1.6 | 1.7 | 4 | 4 |
| 11 | 4 | 4 | 1.1 | 1.3 | 4 | 4 |
| 12 | 4 | 4 | 0.9 | 1.1 | 4 | 4 |
| 13 | 4 | 4 | 0.8 | 1.2 | 4 | 4 |
| 14 | 4 | 4 | 1.0 | 1.2 | 4 | 4 |
| 15 | 4 | 4 | 1.1 | 1.1 | 4 | 4 |
| 16 | 4 | 4 | 0.9 | 1.1 | 4 | 4 |

Note :-*Pseud. = Pseudomonas Staphyl= Staphylococcus Strept.= Streptococcus Data values from 2 averages*

Results of distilled water indicated that *Pseudomonas aeruginosa* and *Bacillus subtillus* were more survival than other tested bacterial isolates until 16 weeks, where the$log_{10}$ reduction were recorded 0.9 and 1.1 cfu/ml, respectively. *Staphylococcus aureus* and *Streptococcus faecalis* reached complete $log_{10}$ reduction at third week. Also, complete $log_{10}$ reduction was obtained at second and fourth week, for *Salmonella* spp. and *E. coli*, respectively (Fig. 1&Fig. 5). At the third week, the lowest values of $log_{10}$ reduction (0.5 cfu/ml)were recorded for *Pseudomonas aeruginosa*, followed by *Bacillus subtillus* (0.6cfu/ml). Moreover, the highest rate of $log_{10}$ reduction occurred in*Salmonella* spp. after one week and *Staphylococcus aureus* or *Streptococcus faecalis* were observed at the second week. Also, in this study, generally the survival from initial counts ($10^4$cfu/ml) for bacterial tested showed a gradual decrease in survival of tested bacteria occurred in time till reaching 16 weeks, except *Pseudomonas aeruginosa* and *Bacillus subtillus* that still living even after 16 weeks. This result meant that *Pseudomonas aeruginosa* and *Bacillus subtillus* were more stable against non-suitable environment (distilled water) than othertested bacteria.




**Note :- *Pseud. = PseudomonasStaphyl= Staphylococcus Strept.= StreptococcusData values from 2 averages***

**Fig. 1:- $Log_{10}$ survival values (cfu / ml) of different bacterial isolates tested in sterile distilled water for 16 weeks at room temperature.**

Data from *E. coli* was agreement with in Northern Ireland researchers (Kerr, etal[6]) observed survival *E. coli* (initial count were about $10^3$ and/or $10^6$) in distilled water at room temperature for 10 weeks. They found that at the initial count $10^3$cfu/ml, the reduction reached 0·64 $\log_{10}$ on day 14, but no bacteria were detected on the third week. While the other bacterial concentration (6 $\log_{10}$cfu/ml) survived at 70 days with $\log_{10}$ reduction 4 cfu/ml.

Also, the present results were in agreement withLiao and Shollenberger,[4] in USA, who observed that *Pseudomonas aeruginosa* (initial log count was $10^8$cfu/ml) were able to survive for 30 weeks (log count was $10^7$cfu/ml)in distilled water at room temperature as well as they reported that *Pseudomonas aeruginosa* remained viable for 12 to 16 years under the same conditions.

Results of *Salmonella* spp. were in line with those obtained by Uyanik, etal[5] in Turkey, who observed that *Salmonella* spp. And *Shigella flexneri* were able to survive in distilled water at room temperature for 5 and 43 days, respectively.

Also, our results were in the same trend with Serrano, etal[7] in Madrid, Spain, who examined three separate strains (*Staphylococcus aureus, E. coli*, and *Pseudomonas aeruginosa*) with initial count $10^6$ cfu/mL in distilled water at room temperature. They observed that all tested strains were reduced by 0.99 $\log_{10}$cfu/ml after 24 hours, except *Pseudomonas aeruginosa* that were able to remain viable for several weeks.

The survival of *Bacillus subtillus* for 16 weeks in distilled water at room temperature in the present investigation was accepted with Friedline, etal [26] in USA as they found that bacterial spores could survive for several years without nutrients in harsh environmental conditions. They also concluded that this may be to water retention inside bacterial spores as well as the presence of protective compounds in the structure of spores (Dipicolinic acid). Moreover, our results concerning Gram-positive cocci were in agreement with Patel, etal[27]in Johannesburg, South Africa, who obtained 84.35% reduction from $10^8$cfu/ml mixed cultures of *Staphylococcus aureus, Enterococcus faecalis* and *Streptococcus mutans* in sterile distilled water at room temperature after 24 hours.

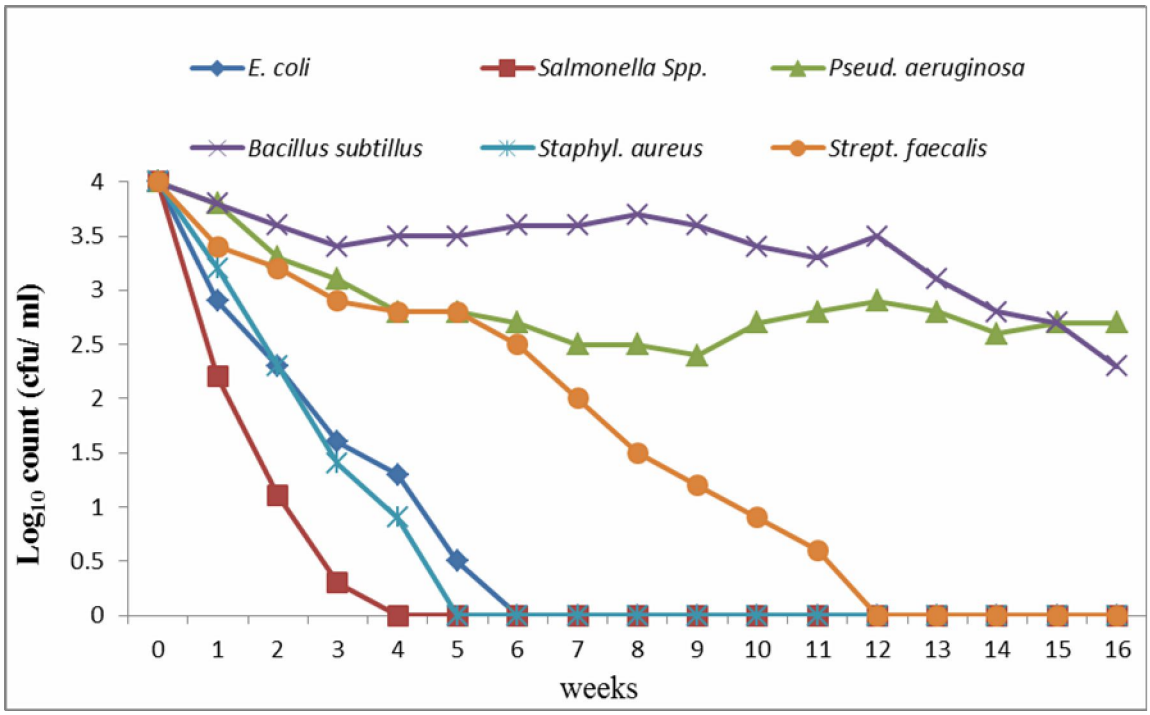
**Survival bacterial isolates in sterile tap water**

Data presented in Table (2) and illustrated by Fig. (2)show the reduction $\log_{10}$ values (cfu/ml) of different bacterial isolates in sterile tap water for 16 weeks at room temperature.

**Table 2:- Reduction $\log_{10}$ values (cfu/ml) of different bacterial isolates in sterile tap water for 16 weeks at room temperature.**

| Incubation time (weeks) | Bacterial isolates | | | | | |
|---|---|---|---|---|---|---|
| | ***E. coli*** | ***Salmonella* spp.** | ***Pseud. aeruginosa*** | ***Bacillus subtillus*** | ***Staphyl. aureus*** | ***Strept.f aecalis*** |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 1.1 | 1.8 | 0.2 | 0.2 | 0.8 | 0.6 |
| 2 | 1.7 | 2.9 | 0.7 | 0.4 | 1.7 | 0.8 |
| 3 | 2.4 | 3.7 | 0.9 | 0.7 | 2.6 | 1.1 |
| 4 | 2.7 | 4 | 1.2 | 0.6 | 3.4 | 1.2 |
| 5 | 3.5 | 4 | 1.2 | 0.5 | 4 | 1.2 |
| 6 | 4 | 4 | 1.3 | 0.4 | 4 | 1.5 |
| 7 | 4 | 4 | 1.5 | 0.4 | 4 | 2.0 |
| 8 | 4 | 4 | 1.5 | 0.3 | 4 | 2.5 |
| 9 | 4 | 4 | 1.6 | 0.4 | 4 | 2.8 |
| 10 | 4 | 4 | 1.3 | 0.6 | 4 | 3.1 |
| 11 | 4 | 4 | 1.2 | 0.7 | 4 | 3.7 |
| 12 | 4 | 4 | 1.1 | 0.5 | 4 | 4 |
| 13 | 4 | 4 | 1.2 | 0.6 | 4 | 4 |
| 14 | 4 | 4 | 1.4 | 0.9 | 4 | 4 |
| 15 | 4 | 4 | 1.3 | 0.6 | 4 | 4 |
| 16 | 4 | 4 | 1.3 | 1.7 | 4 | 4 |

Note :-*Pseud. = PseudomonasStaphyl= Staphylococcus Strept.= StreptococcusData values from 2 averages*




**Note :-*Pseud. = Pseudomonas Staphyl= Staphylococcus Strept.= Streptococcus Data values from 2 averages***

**Fig. 2:- $Log_{10}$ survival values (cfu/ml) of different bacterial isolates tested in sterile tap water for 16 weeks at room temperature.**

Results revealed that most of the tested bacterial isolates (*Staphylococcus aureus, Streptococcus faecalis, E. coli and Salmonella* spp.)were died during incubation for 16 weeks in sterile tap water at room temperatures, while *Pseudomonas aeruginosa,* and *Bacillus subtillus* were still alive even after 16 weeks of incubation in sterile tap water at room temperatures. The present data showed that bacterial $log_{10}$ reduction counts at the end of the experiment (16 weeks) were 1.3 and 1.7cfu / ml for *Pseudomonas aeruginosa,* and *Bacillus subtillus,* respectively. On the other hand, the complete $log_{10}$ reduction was observed at $6^{th}$, $4^{th}$, $5^{th}$ and $12^{th}$ week for *E. coli*, *Salmonella* spp., *Staphylococcus aureus* and *Streptococcus faecalis*, respectively(Fig. 5). With regard to the present results, the rate of death for *Pseudomonas aeruginosa* and *Bacillus subtillus* were 32.5 and 42.5%, respectively.

Similar studies in Turkey, it was observed that *Salmonella typhi* and *Shigella flexneri* could survive in tap water for 29 and 57 days at room temperature, respectively (Uyanik, etal[5]).

Also, in Ghana, the viability of total coliform, *E. coli* and Heterotrophic Plate Count (HPC) bacteria were tested in sterile tap water for 21 days at room temperature (25ºC) and 37ºC (Sakyi and Asare,[10]). A complete $log_{10}$ reduction occurred at the $7^{th}$ day for both total coliform and *E. coli* at all incubation temperatures, while only 28.6 and 32.6% of tested HPC bacteria incubated at 25 and 37ºC incubations (at the $7^{th}$ day), respectively, but died them at the $21^{th}$ day of the experiment. It was concluded that prolonging of bacterial survival depended on incubation temperature and the nutrient in aquatic environment (Laurent, etal [28] and Prevost,etal [29]).

Results of spore formers (*Bacillus subtillus*) in the present study were line with Brillard, etal[30], in France who found that only 24.7% of spore formers (*Bacillus cereus*) died after 50 days of incubation at 25ºC in sterile drinking water. It was concluded that *Bacillus subtillus* can survive under hard conditions for a long period because of its ability to transform to resistant spores. Moreover, prolonged survivalof spore formers might be due to the compound structure of the spore thus preventing loss of its water content as well as the presence of protective compounds (such as Dipicolinic acid) in the spores (Anthony, etal[8]).

On the contrary, Grandjean, etal[31] in France found no difference in the initial $log_{10}$ count ($log_{10}$ 5 cfu/ml) of *E. coli* incubated in sterile drinking water at 25ºC before and after 21 days of incubation, indicating that neither growth nor lysis had occurred.

But, results in this investigation of *E. coli* and *Salmonella* spp. in the same trend with Djaouda, etal[12] in Cameroon, the initial counts of *E. coli* and *Salmonella* spp. cells (3 $Log_{10}$cfu/ml for each) were stored in sterile drinking water for 3 days at room temperature (30±2 ºC). After incubation period, counts were lowered to levels 1 and 1.61 $log_{10}$cfu/ml, respectively.

Results of *Staphylococcus aureus* and *Pseudomonas aeruginosa* in the present work were in accordance with Serre, etal[32] in France, the viability of *Staphylococcus aureus* and *Pseudomonas aeruginosa* (with 3 different $log_{10}$ counts of 7, 5 and 3cfu/ml for each) were tested in sterile tap water stored at room temperature (20 to 25°C) for 6 months. The obtained results showed complete $log_{10}$ reduction of *Staphylococcus aureus* after 9, 3 and 2 days of incubation, respectively, while *Pseudomonas aeruginosa* reached to 7.1, 5.8 and 5.3 cfu/ml, respectively after 6 months of incubation. Moreover, authors concluded that *Pseudomonas aeruginosa* could survive more than 40 months in tap water.

Concerning *Streptococcus faecalis,* results of the present study were in line with Mcfeters, etal[33] in Montana State who recorded about one $log_{10}$ reduction of fecal streptococci in sterile water from $10^4$cfu/ml after 3 days. Also inAustralia, it was found that *Streptococcus faecalis* (in a concentration of $10^7$cfu/ml) in sterile water at room temperature showed 3 $log_{10}$ reduction after 58 days post inoculation (Davies, etal[34]).

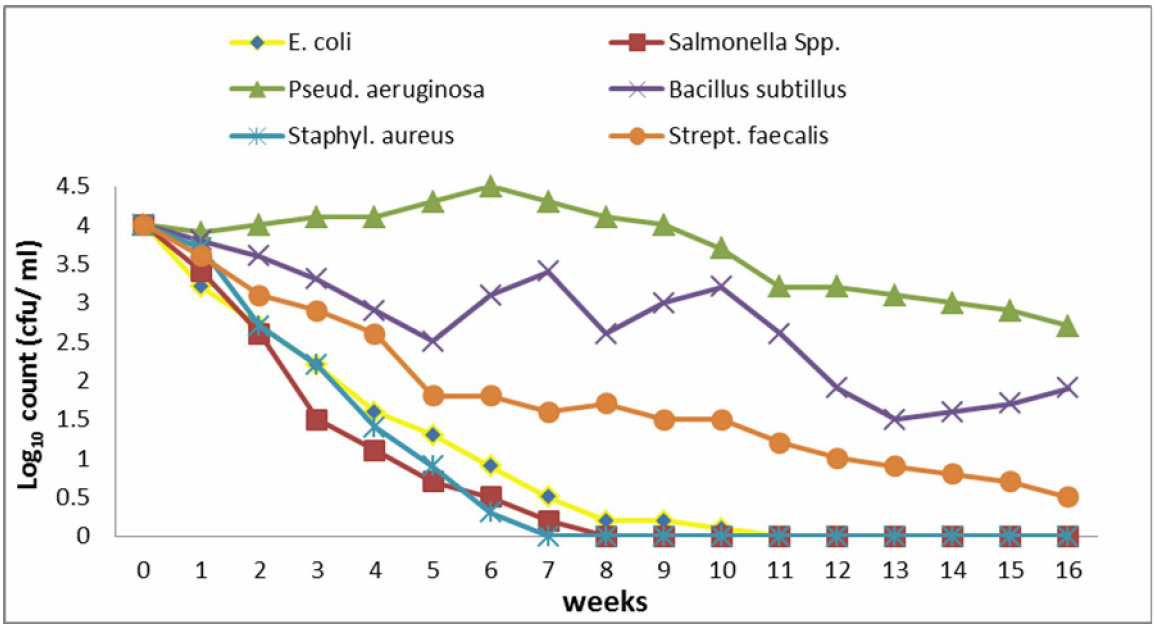
In general, the survival of bacterial cells in drinking water depended on sources of the tested bacterial isolates, type and counts of bacteria, characters and pH water as well as incubation temperature and time (Laurent,etal[28]; Prevost,etal[35]; Serre, etal[32];WHO[2], and Jenkins, etal[36]).

**Survival bacterial isolates in groundwater**

**Table 3:- Reduction $log_{10}$ values (cfu/ml) of different bacterial isolates in sterilized groundwater for 16 weeks at room temperature.**

| Incubation time (weeks) | Bacterial isolates | | | | | |
|---|---|---|---|---|---|---|
| | *E. coli* | *Salmonella* spp. | *Pseud. aeruginosa* | *Bacillus subtillus* | *Staphyl. aureus* | *Strept.f aecalis* |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 0.8 | 0.6 | 0.1 | 0.2 | 0.3 | 0.4 |
| 2 | 1.3 | 1.4 | 0 | 0.4 | 1.3 | 0.9 |
| 3 | 1.8 | 2.5 | -0.1 | 0.7 | 1.8 | 1.1 |
| 4 | 2.4 | 2.9 | -0.1 | 1.1 | 2.6 | 1.4 |
| 5 | 2.7 | 3.3 | -0.3 | 1.5 | 3.1 | 2.2 |
| 6 | 3.1 | 3.5 | --0.5 | 0.9 | 3.7 | 2.2 |
| 7 | 3.5 | 3.8 | -0.3 | 0.6 | 4 | 2.4 |
| 8 | 3.8 | 4 | -0.1 | 1.4 | 4 | 2.3 |
| 9 | 3.8 | 4 | 0 | 1 | 4 | 2.5 |
| 10 | 3.9 | 4 | 0.3 | 0.8 | 4 | 2.5 |
| 11 | 4 | 4 | 0.8 | 1.4 | 4 | 2.8 |
| 12 | 4 | 4 | 0.8 | 2.1 | 4 | 3 |
| 13 | 4 | 4 | 0.9 | 2.5 | 4 | 3.1 |
| 14 | 4 | 4 | 1 | 2.4 | 4 | 3.2 |
| 15 | 4 | 4 | 1.1 | 2.3 | 4 | 3.3 |
| 16 | 4 | 4 | 1.3 | 2.1 | 4 | 3.5 |

Note :-*Pseud. = PseudomonasStaphyl= Staphylococcus Strept.= StreptococcusData values from 2 averages*




**Note:-*Pseud* = *Pseudomonas*, *Strept.*= *Streptococcus*, *Staphyl.* = *Staphylococcus*.**
***Data values from 2 averages***
**Fig. 3:- $Log_{10}$survival values (cfu/ml) of different bacterial isolates tested in sterile groundwater for 16 weeks at room temperature.**

The $log_{10}$ survivalof different bacterial isolates tested in sterilized groundwater for 16 weeks at room temperature were showed in Table 3 and Fig. 3.

Results showed that *Pseudomonas aeruginosa, Bacillus subtillus* and *Streptococcus faecalis* survived for more than 4 months but complete $log_{10}$ reduction were observed for *E. coli, Salmonella* spp. and *Staphylococcus aureus* (Fig. 5) in sterilized groundwater**.** Moreover, the $log_{10}$ reduction reached 1.3, 2.5 and 3.5 cfu/ml for *Pseudomonas aeruginosa, Bacillus subtillus* and *Streptococcus faecalis,* respectively, (Table 3)at the sixteenth week. Complete $log_{10}$ reductions were observed at the $11^{th}$ , $8^{th}$ and $7^{th}$ week for *E. coli, Salmonella* spp. and *Staphylococcus aureus*, respectively, (Fig. 5). On the other side, the $log_{10}$ number of *Pseudomonas aeruginosa* cells increased in concentration from the third week until eighth week with a range of 0.1 to 0.5 cfu/ml and then decreased to be 1.3 cfu/ml at end of the experiment (16 weeks). Results revealed that $log_{10}$ counts of *Pseudomonas aeruginosa* increased then decreased due to some factors such as pH, temperatures, nutrients chemical contents of groundwater (Laurent,etal[28] and Prevost, etal[35]).

In a study in USA, the viability of *Salmonella typhimurium* and *Streptococcus faecalis*(with initial $log_{10}$ counts 7.33 and 5.6 cfu/ml, respectively) was testedin sterilized groundwater at 22°C for up to 15 days (Bitton, etal[37]). It was found that the count of *S. typhimurium* decreased more than *S. faecalis* with $log_{10}$ reduction 2.13 and 0.4 cfu/ml, respectively after incubation period, indicating that streptococci were more resistant than salmonellae group for survival in aquatic environment.

Results in the study were in line with Filip, etal[38] who followed count(initial count between $log10^6$ to $10^7$) of some pathogenic bacteria for 100 days in sterilized groundwater at room temperature. They observed that approximately inactivation rates ($log_{10}$/day) reached0.6, 0.36, 0.2, 0.04, 0.03 and 0.01 for *B. megaterium, B. cereus, S. aureus*, *S. typhimurium*, *E. coli* and *S. faecalis,* respectively. In addition, these authors found that in spite of the $log_{10}$ reduction of *Bacillus* spp. recorded high rate of reduction compared with other tested bacteria even after period of experiment (100 days). In addition, from the authors' original bacteria which mentions previous were survival during this work and some of them were survived more than this time. Moreover, the same authors noticed that counts of *Pseudomonas aeruginosa* increased till the $11^{th}$ day, then decreased but they were still alive over 100 days.

Other workers in Egypt (El-Leithy etal[39]) showed that survival of *E. coli* was in line with our results, but they used initial $log_{10}$ count 6 cfu/ml of *E. coli* (strain O157:H7 ; ATCC 35150) at room temperature (20 ± 2°C) in sterilized groundwater. They found that complete $log_{10}$ reduction occurred at the $84^{th}$ day which was

longer than our result (the 77th day) that might be due to difference in initial count. The presence of *E. coli* in groundwater is of a major water quality concern and consequently public health hazards (Pandey, etal[29]).

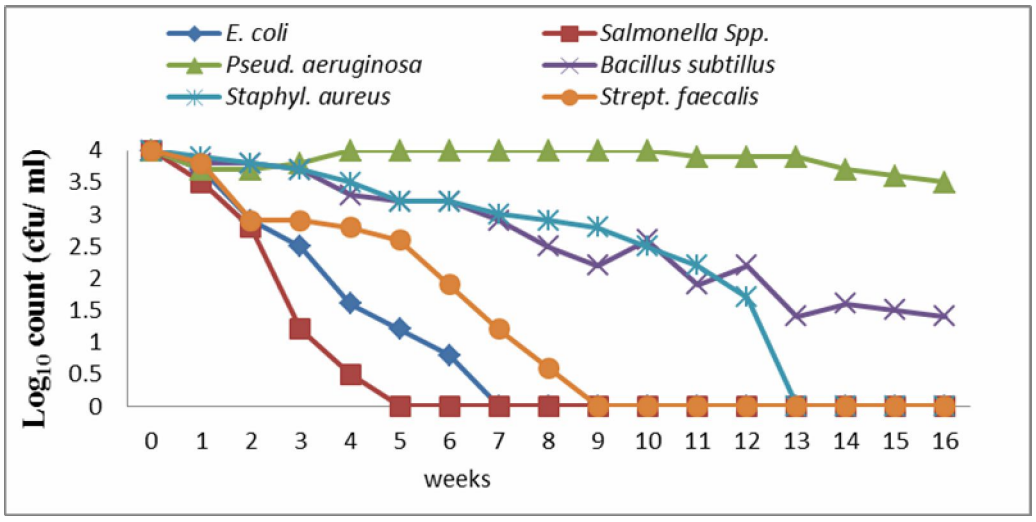
In addition, other workers reported that *Pseudomonas aeruginosa, S.aureus* and *Bacillus* spp. could survive in groundwater for several months or several years as these bacteria have the ability to re-grow in unsuitable water environment (Warburton, et al[40]; 1986;Lechevallier, etal[41] and John & Rose[16]).

**Survival bacterial isolates in seawater**

**Table 4:- $log_{10}$ Reduction values (cfu/ml) of different bacterial isolates in sterilized seawater for 16 weeks at room temperature**

| Incubation time (weeks) | Bacterial isolates | | | | | |
|---|---|---|---|---|---|---|
| | *E. coli* | *Salmonella* spp. | *Pseudomonas aeruginosa* | *Bacillus subtillus* | *Staphyllococcus aureus* | *Streptococcus faecalis* |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 0.2 | 0.5 | 0.3 | 0.2 | 0.1 | 0.7 |
| 2 | 1.1 | 1.2 | 0.3 | 0.2 | 0.2 | 1.1 |
| 3 | 1.5 | 2.8 | 0.2 | 0.3 | 0.3 | 1.1 |
| 4 | 2.4 | 3.5 | 0 | 0.7 | 0.5 | 1.2 |
| 5 | 2.8 | 4 | 0 | 0.8 | 0.8 | 1.4 |
| 6 | 3.2 | 4 | 0 | 0.8 | 0.8 | 2.1 |
| 7 | 4 | 4 | 0 | 1.1 | 1 | 2.8 |
| 8 | 4 | 4 | 0 | 1.5 | 1.1 | 3.4 |
| 9 | 4 | 4 | 0 | 1.8 | 1.2 | 4 |
| 10 | 4 | 4 | 0 | 1.4 | 1.5 | 4 |
| 11 | 4 | 4 | 0.1 | 2.1 | 3.1 | 4 |
| 12 | 4 | 4 | 0.1 | 1.8 | 3.4 | 4 |
| 13 | 4 | 4 | 0.1 | 2.4 | 4 | 4 |
| 14 | 4 | 4 | 0.3 | 2.4 | 4 | 4 |
| 15 | 4 | 4 | 0.4 | 2.5 | 4 | 4 |
| 16 | 4 | 4 | 0.5 | 2.6 | 4 | 4 |

Note :-*Pseud. = Pseudomonas,Staphyl= Staphylococcus*
*Strept.= Streptococcus* *Data values from 2 averages.*




**Note:-*Pseud = Pseudomonas, Strept.= Streptococcus, Staphyl. = Staphylococcus***
***Data values from 2 averages.***
**Fig. 4:- $Log_{10}$survival values (cfu/ml) of different bacterial isolates tested in sterile seawater for 16 weeks at room temperature.**

Data presented in Table (4) and illustrated by Fig. (4)show that the $log_{10}$ reduction of cell forming unit (cfu)/ml for total viable bacteria for 4 months in sterilized seawater at room temperature. Complete $log_{10}$ reductions were detected after seven, five and nine weeks of incubation for *E. coli*, *Salmonella* spp. and *Streptococcus faecalis*, respectively,(Fig. 5). On the other hand, *Pseudomonas aeruginosa*, and *Bacillus subtillus* showed 0.5 and 2.6cfu/ml $log_{10}$ reductions, respectively. Moreover, during this investigation, it was noticed that the rate of $log_{10}$reduction of *Pseudomonas aeruginosa* cells throughout the whole experiment was very low compared with other tested bacteriareaching2.5%. Generally, the ability of tested bacteria to survive in seawater decreased in an ascending order for *Salmonella* spp., *E. coli*, *Streptococcus faecalis*, *Staphylococcus aureus, Bacillus subtillus,* and *Pseudomonas aeruginosa*.

In a study conducted in Barcelona, Spain, the survival of 2 strains of *E. coli* (one adapted and other non-adapted) in sterilized seawater with initial count $10^7$ cfu/ml at room temperature ($20^0$C) was tested (Garcia-Lara, etal[42]). Their result showed that no change in $log_{10}$ reduction of adapted strain, while other strain showed 4 $log_{10}$ reduction after 30 days of incubation. This difference in behavior of the 2 strains might be due to difference of sources of isolates (John, and Rose,[16]).

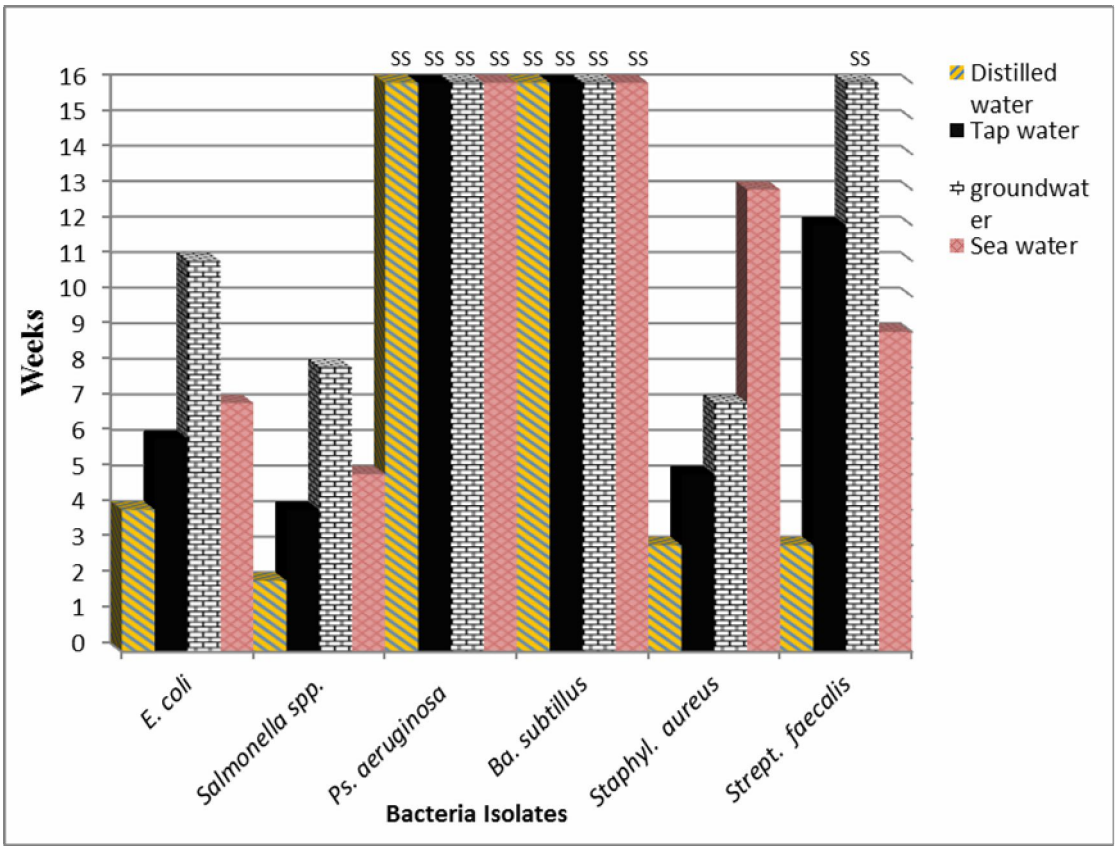
In Swedenthe survival of *Salmonella enterica* (initial counts were $10^7$cfu/ml) in sterilized seawater at 18 $^0$C for 8 weeks was studied (Hernroth, etal[21]). At the end of the experiment, counts of *Salmonella enterica* reached $1.5x10^3$cfu/ml, while in the present investigation *Salmonella* spp. were disappeared completely after 5 weeks of incubation. However, the survival of bacterial cells in seawater depended on many factors such as pH, sunlight, incubation temperatures and the physic-chemical characters of seawater as well as competition between microorganisms (John and Rose[16])..

Concerning *Pseudomonas aeruginosa,* a study was conducted in Spain for testing the viability (with initial count $10^7$cfu/ml)in natural untreated seawater, and treated seawater (filtered) for 20 days at room temperature (Cornax, etal[43]). On daily record, it was found that in untreated seawater samples all *Pseudomonas aeruginosa* bacteria died-off at the $14^{th}$ day. In our opinion, this might be due to competition for survival between microorganisms and presence of toxic substance. On the other hand, in treated seawater *P. aeruginosa* decreased by about $log_{10}$ reduction 0.5 cfu/ml after 20 days (end of the experiment). These data might be explained by Khan, etal[44], in Japan who concluded that *Pseudomonas aeruginosa* can tolerate different harsh conditions in marine water for several weeks or months such as different incubation temperatures (−20, 0, 4, 25 and 37°C), NaCl concentrations (0 to 7% [w/v]) and pH (4.0 to 9.0).

Data of *Staphylococcus aureus* in the present work were in line with Tolba, etal[45] who tested the viability of this bacteria in sterile seawater and river water at ambient temperature after 14 days post inoculation ($10^5$cfu/ml). They observed that the log count of *S. aureus* in seawater reached $10^3$cfu/ml, while these bacteria died off in river water. In another study, the decaying rate of *S. aureus* in seawater for 14 days at room temperature (20°C) was ~ 28%, while in freshwater it was ~34–44% (Levin-Edens, etal[46]). These data were in agreement with our study where the survival of *S. aureus* in seawater was more than river Nile (freshwater).

In a study conducted in Italy, the survival of *Streptococcus faecalis* was not in line with that in the present study (Figure 4) as *Streptococcus faecalis* bacteria were non-culturable after 42 days incubation in sterile lake water at room temperature (25°C) for 56 days (Lieo,` etal[47]). They concluded that some species lose culture-ability rapidly but others are able to survive in seawater for several months.

Also, In a study on *Bacillus thuringiensis*, result of current work agreed with that of Furlaneto, etal[48] who demonstrated that no change in log count of *Bacillus* cells (vegetative or spores) (approximately $10^8$ cfu/ml) kept in sterile lake water at 30°C for 11 days. Moreover, Sinclair, etal[49]reported that vegetative cellsof *Bacillus* spp. can be able to survive in sea water for 20 months, but spores can survive for several years.




**Note:-*Pseud* = *Pseudomonas*, *Strept.*= *Streptococcus*, *Staphyl.* = *Staphylococcus***
***SS* = *Still survived after 16 weeks***
**Fig. (5) The week number at complete $log_{10}$ reduction of different bacterial isolates tested in different water sources at room temperature.**

## Conclusion

Complete $log_{10}$ reductions were observed for *E. coli*, *Salmonella* spp., *Staphylococcus aureus* and *Streptococcus faecalis* in sterilized waters (distilled water, tap water and seawater). On the contrary, complete $log_{10}$ reductions were not detected for all types of tested bacteria in sterilized groundwater samples. By the end of the experiment, *Salmonella* spp.,*E. coli* and *Staphylococcus aureus* as well as *Streptococcus faecalis* disappeared completely in sterilized distilled water.

On the contrary, *Pseudomonas aeruginosa* and *Bacillus subtillus* were still alive in all types of examined water even after the end of the experiment.

*Staphylococcus aureus* survived in seawater more than *Streptococcus faecalis*, while *Streptococcus faecalis* survived more than *Staphylococcus aureus* in groundwater.

In general, the survival time of *E. coli* was longer than that of *Salmonella* spp. in different examined water sources. Consequently, Gram-positive bacteria survived for longer times than Gram-negative bacteria in different water sources.

Survival of bacteria in Egyptian aquatic environment depended on some factors such as types of bacteria and physic-chemical criteria of water. The presence of bacteria in water sources used in different human activities has negative effects on public health.

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