Original Research Article

Detection of Burkholderia cepacia in pharmaceutical products

Marina Ramsis Fahmy1,* , Youssry El-Sayed Saleh2 , Mary Sobhy Khalil2

1 Pharmaceutical Company, Cairo, Egypt
2 Dept. of Botany and Microbiology, Faculty of Science, Cairo University, Egypt

ABSTRACT

Introduction: Burkholderia cepacia complex (BCC) have recently received a reasonable attention as one of the major risks in susceptible pharmaceutical products. This microorganism can easily spread and cause severe contamination especially to the water stations for pharmaceutical companies. It rapidly grow within the product and cause cystic fibrosis and septicemia in humans.

Aim: The traceability of the sources of contamination of pharmaceutical non-sterile aqueous preserved products and how to control it during the manufacturing operations as it is concern with the public health.

Materials and Methods: The samples collected for the analysis were taken from different sources provided from the pharmaceutical company such as: aqueous finished products, filling machines, preparation tanks and water stations, identified by API 20NE, Vitek 2 compact system and 16S rRNA.

Results: From 213 different samples of finished product, 22 bacterial isolates had been identified as Burkholderia cepacia group. Raw materials and primary packaging materials did not result in any bacterial isolates while the machine surfaces and preparation tanks were contaminated. From 384 pharmaceutical water samples, 35 isolates were identified as B. cepacia.

Conclusion: Our study suggests that the chlorine treatment and hydrogen peroxide have a significant effect on B. cepacia in water disinfection.

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1. Introduction

Burkholderia cepacia was identified as Pseudomonas cepacia before 1992 as the cause of onion rot (cepacia in Latin = onion).1,2 B. cepacia is Gram-negative bacilli, 1-5 μm in length and 0.5-1.0 μm in width, aerobic, free-living, motile non-lactose fermenting and produce catalase.3 The genus Burkholderia belongs to the β-subdivision of the phylum Proteobacteria. Since the genus name was first assigned, the taxonomy of the genus Burkholderia has undergone considerable changes and the genus now includes 22 validly described species: B. cepacia (the type species), Burkholderia caryophylli, Burkholderia mallei, Burkholderia pseudomallei, Burkholderia gladioli, Burkholderia plantarii, Burkholderia glumae, Burkholderia vietnamiensis, Burkholderia andropogonis, Burkholderia multivorans, Burkholderia glathei, Burkholderia pyrrocinia, Burkholderia thailandensis, Burkholderia graminis, Burkholderia phenazinium, Burkholderia caribensis, Burkholderia kururiensis, Burkholderia ubonensis, Burkholderia caledonica, Burkholderia fungorum, Burkholderia stabilis and Burkholderia ambifaria.4 The mortality rate of the patients infected by cystic fibrosis due to BCC is high percentage as 62-100%.5 B. cepacia is an opportunistic pathogen, it is one of the most frequently isolated bacterial contaminants in pharmaceutical samples around the world.2,6-8 B. cepacia is able to survive and multiply in the presence of disinfectants.9

Recently, Food and Drug Administration10 warned drug manufactures that Burkholderia cepacia can remain alive in non-sterile aqueous products leading to the development of resistance to preservative systems used to protect product formulations. Its detection in pharmaceutical manufacturing
process is difficult and products recalls are recurrent. The most frequent reasons of contamination of pharmaceutical products include microbial contaminant of the water used in product development contaminated and poor preservative system. Pharmaceutical grade water is critical material for equipment cleaning, as well as, ingredient water in drug products.

The aim of this research is to trace the sources of contamination of pharmaceutical non-sterile aqueous preserved products and how to control it during the manufacturing operations.

2. Materials and Methods

2.1. Isolation

The samples collected for the analysis were taken from different sources provided from the pharmaceutical company such as:

a) A aqueous finished products, 213 batches were produced in 2 years. During this time, 22 bacterial isolates were sub-cultured and identified. The test method involves transferring 10 ml from the test sample to (90) ml sterile Tryptone Azolectin Tween (TAT) broth medium, 10 ml of the prepared sample were filtered, cultured on Tryptic Soy Agar (TSA) medium and incubated at 30°C -35°C for 3-5 days and another 10 ml were aseptically added to about 100 mL sterile trypticase soy broth containing 4% Tween 20 (TSBTW). After incubation at 30°C -35°C for 18-24 hours, 0.1 ml of TSBTW were transferred to the surface of Oxidation-Fermentation-Polymyxin-Bacitracin-Lactose agar medium (OFPBL) and Burkholderia cepacia selective agar (BCSA) agar plates inverted and incubate d at 30°C -35°C for 18-72 hours.

b) From filling machines and preparation tanks, before each prepared batch we sampled the final rinse from 10 points. The method of testing was membrane filtration and the agar used was Reasoner’s 2A agar medium (R2A) incubated for 5 days at 30°C-35°C.

c) Water system including water treatment station and Reverse Osmosis (R.O.) water station, the samples were collected for one year. During this time, 384 samples were tested, from these 35 samples showed growth were sub-cultured and identified. The test method used is membrane filtration technique.

2.2. Identification

Smears of fresh isolates were picked on slides and heat fixed and examined microscopically and by the routine bacteriological methods including: subculture in selective media, biochemical tests: indole test, oxidase test, catalase test, gelatin hydrolysis, Voges-Proskauer test, methyl red test, carbohydrate fermentation test, API 20 NE and VITEK 2 system.

2.3. Disinfectant efficacy test

Using two disinfectants such as Tego (alkyl diazapetane) and Viragri plus (blend of glutaraldehyde and quaternary ammonium compound) against B.cepacia after contact time 15 minutes on 4 different surfaces (Ceramic, Epoxy, Stainless-steel and glass).

2.4. Susceptible odd product

The current product formula contain the preservative methyl paraben 0.1%, propyl paraben 0.02% and cremophore 1%. The proposed formula are the same concentration of parabens but different concentrations of cremophore. These dilutions are inoculated with a known volume not more than 100 CFU/ml of Burkholderia cepacia.

2.5. Antimicrobial preservative efficacy test

Two modified formula from this product were proposed and the effect of their preservatives activity were tested against B.cepacia: Formula X: 0.25% cremophore, no ethanol with parabens and Formula Y: 5% ethanol with parabens.

2.6. Molecular characterization using 16S rRNA.

The 16S rRNA gene sequences of the studied bacteria was sequenced in Sigma Lab after which the sequences were blasted to GenBank database at the National center for Biotechnology Information (NCBI) and a phylogenetic tree was illustrated using Clustal omega website.

3. Results

3.1. Stage I

21 batches from aqueous finished products were produced, 19 batches out of 21 were rejected due to their contamination as shown in Table 1.

The 19 samples were negative indole, positive catalase, negative gelatinase, negative VP, negative MR and positive oxidase except isolates (3F, 7F, 10F, 14F and 19F).

The contaminant organism isolated from 19 samples had the ability to ferment certain carbohydrates such as glucose, cellobiose, mannitol, mannose, sucrose and lactose and change the medium color from red into yellow due to acid production while the other carbohydrates such as arabinose, maltose and sorbitol cannot ferment them and the medium color was not changed.

The contaminant organism isolated identified as “Burkholderia cepacia” by using identification systems as API 20 NE strip and VITEK 2 system.

3.2. Stage II

a)- Bacterial samples obtained from tanks and machine filling.
Before each prepared batch we sampled the final rinse from 10 points (syrup tank – hold tank – buffer tank – pipe – 6 nozzles) so we have 210 samples depending on number of prepared batches in Stage I (21 F).

190 samples out of 210 were contaminated and identified as the same contaminant organism. “Burkholderia cepacia” as in the aqueous finished products in Stage I although the washing and sanitization of these tanks and nozzles by ethyl alcohol 70%.

After these important findings, we conclude that the source of contamination by a percentage 90% is the machine preparation and hold tanks and the machine filling pipes and nozzles.

We should disinfect all of these sites by a new disinfectant which has a strong bactericidal effect especially on Burkholderia cepacia.

b) Disinfectant efficacy test

Tego (Alkyl diazapetane) is used for the disinfection of floors, walls, work surfaces and process equipment and it is applied to the surface.

As the log reduction is more than 3 as the disinfectant Tego 2000 has a great bactericidal and fungicidal effect against organisms at 15 minutes on all surfaces, so it is recommended for using this disinfectant instead of ethyl alcohol 70% (Table 2).

Viragri plus is based on an optimized blend of glutaraldehyde and quaternary ammonium compound (QAC) in aqueous solution. It is a highly effective, non-oxidizing disinfectant specially formulated for use on cleaned surfaces in the sectors where biocidal activity is required like pharmaceutical industry applications.

As the log reduction is more than 3 as the disinfectant Viragri plus has a great bactericidal and fungicidal effect against organisms at 15 minutes on all surfaces (Table 3).

At the end of this stage we conclude that the rinsing with these two disinfectants on all the machine tanks and filling parts with planned rotation instead of ethyl alcohol 70% only will be the efficient solution to prevent the recurrence of all the contamination by Burkholderia cepacia.

Accordingly monitoring and examining the produced batches after this treatment is very important as shown in the third stage.

3.3. Stage III

3.3.1. Bacterial isolation from aqueous finished products.

In this Stage, after the examination of 93 samples from different products, the results showed that 90 syrup batches (samples) were clean, free from the contaminant organism and accepted for their release in the market, this is high percentage of success 96.7%. While a 3 samples (22F, 23F, 63F) showed yellow colonies on TSA medium, knowing that these 3 samples were from the same product.

3.3.2. Susceptible odd product

It was shown in Table 4 that as the concentration of cremophore decrease the bacterial count also decrease and the use high concentration of cremophore 1% in the current formula hide the effect of preservatives which is susceptible to the contamination.

Consequently it was proposed to the Research and Development Department to prepare a trial batch from this product containing 0.25% of cremophore instead of 1%.

3.3.3. Antimicrobial preservative efficacy test

The results of these experiments in Figure 1 showed that the formula X could not be contaminated with Burkholderia cepacia as compared with formula Y. The new formula X should be produced instead of current one to prevent the recurrence of contamination.

3.4. Stage IV

3.4.1. Bacterial Isolation from water system

From R.O. station, out of 144 samples, 13 samples (from return point) 9.02% were identified as “Burkholderia cepacia group” the rest were free from contamination.

From water treatment station, out of 240 samples, 22 samples (from storage tank) 9.16% were identified as “Burkholderia cepacia group” while the rest were free from contamination.

As the contaminant organism is the same isolated from finished products, machines and water stations, conclusively the main root cause of contamination was the WATER.

3.4.2. Bacterial Isolation from aqueous finished products

All the 99 produced batches were pure and show no growth on selective medium.

As conclusion after the water treatments, 100% of the finished products were clear, accepted, free from any contamination especially Burkholderia cepacia group and returned to the markets safely.

Molecular characterization 16S rRNA gene sequence of the studied bacteria sequenced as Burkholderia cepacia complex. Sequences were submitted to GenBank database at NCBI under accession number: MN610444. Phylogenetic relationship of this species was blasted with other closely related bacterial species present in GenBank and a phylogenetic tree was illustrated as shown in Figure 2.

4. Discussion

Quality of pharmaceutical product were greatly affected by environmental factors during manufacturing operations. It is necessary to control all these factors including raw materials, primary packaging materials, preparation tanks and filling machines, water system and the finished product to achieve the quality standards.
Aqueous finished products such as syrups provide a suitable environment for the growth and survival of both pathogenic and non-pathogenic microorganisms. Bacterial contamination of drug manufacturing industry affect odour, colour, taste of the products thus rendering them unacceptable to the patients. Two years ago, investigations showed that Burkholderia cepacia is a major contaminant in both sterile and non-sterile products in hospitals and pharmaceutical products. A review of U.S. FDA recall data from January (2012) to July (2012) found that 39% of contamination cases in non-sterile products were due to the presence of B. cepacia.

The authors found an overwhelming bacterial contaminant in pharmaceutical products in the end of stage I and identified by API NE20 as Burkholderia cepacia and identified by VITEK 2 as Burkholderia cepacia group. Furthermore, when the isolate identified at its molecular level by 16S rRNA gene sequences techniques, the result obtained showed that this bacteria is Burkholderia cepacia complex (BCC).

High similarity of B. cepacia complex (BCC) (typically above 98%) are measured, indicating that BCC species are phylogenetically very closely related.

Several studies have indicated problems with misidentification of Burkholderia species using phenotypic methods. Recent taxonomic advances have demonstrated that B. cepacia is actually a cluster of at least seven closely related genomic species (or genomovars) now called the B. cepacia. Genomovars II, IV and V are now formally named Burkholderia multivorans, Burkholderia stabilis and Burkholderia vietnamiensis, respectively (with the species designation B. cepacia being reserved for genomovar I). All of which may cause infections among immunocompromised patients and other vulnerable individuals. A combination of phenotypic and molecular tests such as 16S rRNA are recommended for differentiation among the genomovars of the Burkholderia cepacia complex.

Burkholderiaceae contributed by more than 80% to the major hazard that could be delivered to patients through drugs, and this high percentage is in line with results found by the authors in Stage I.

Burkholderia cepacia is a clear pathogen its infection can lead to lung dysfunction and death.

To address these concerns, pharmaceutical and healthcare manufacturers initiate procedures to prevent contamination of non-sterile drug products such as sanitary design, equipment cleaning and environmental monitoring.

The most probable root causes of contamination with Burkholderia cepacia in pharmaceutical industries are absence of cleaning validation studies, poor water system control and design, inadequate microbiological controls and using one type of disinfectant for long period of time.

Excessive bacterial samples isolation from different sources run from the raw material, the packaging materials of these products and finally the machine preparation tanks and filling nozzles to investigate the source of contamination in aqueous products isolated in Stage I.

The results obtained in Stage II showed that the raw materials and primary packaging materials are not the source of contamination while the preparation tanks and machines filling are the source. Consequently, the authors doubted in the disinfectant used in machines cleaning procedures which was only ethyl alcohol 70%.

These results were in agreement with about the causes of contamination with B. cepacia in pharmaceutical companies, insufficient cleaning procedures emphasizing the contamination of the preparation tanks and machines filling in addition the use of one type of disinfectant for long period of time was also a cause of contamination because unfortunately B. cepacia able to survive and multiply in the presence of disinfectants.

Many pharmaceutical manufactures will routinely use two “in-use” disinfectants in a specified rotational sequence for the site disinfection program.

The authors performed a disinfectant efficacy test of other non-oxidizing disinfectants as Tego (alkyl diazepane) and Viragri (quaternary ammonium compounds) against the contaminant organism B. cepacia on different surfaces and different contact time as showed in Stage II.

The authors succeeded in providing finished products clean from the contaminant organism by a percentage reached to 96.7% after performing the rotation plan between all of these tested disinfectants for one year as shown in Stage III, but 3.3% were still contaminated by B. cepacia, knowing that this percentage was from the same susceptible contaminant product. So, the authors studied this product ingredients, its preparation and its formulation and found the presence of methyl paraben and propyl paraben as preservative agents, cremophore act as solubilizing agent and some sweetening agents as sorbitol and glycerin. These findings are in line with who said that the most common method for countering microbial contamination is the use of preservatives.

The capability of Burkholderia cepacia to grow in well preserved formulation requires that the organism does not corrupt the shelf life stability of the product. If an organism exists at small concentration at manufacture the non-sterile product and this organism has the capability to multiply in the product, its potential to degrade the shelf-life of the product is clear. This is certainly a real concern with Burkholderia cepacia and a prudent activity might well be challenge the preservative system of the non-sterile medication with this organism in addition to the compendial organism ordinarily used within antimicrobial effectiveness test.

Pharmaceutical preparations are especially susceptible to microbial growth since of the nature of their ingredients. Such preparations are protected by the addition of anti-
microbial agents in the formulation to devastate and repress the growth of those microorganisms that may contaminate the product during manufacturing or use. Among the most commonly used additive in the preservation of aqueous pharmaceutical preparations are methyl paraben because of their synergistic impacts. Parabens have been used as preservative for over 70 years.  

In the present study, the authors revealed finally that the cause of product susceptibility to contamination, the high concentration of cremophore (1%) hide the effect of preservatives. A modification in formula was suggested such as: Formula X: 0.25% cremophore instead of 1% with parabens and Formula Y: 5% ethanol with parabens. In Stage III these two formulas in addition to the current one undergo an antimicrobial preservative efficacy test especially against B.cepacia. Consequently the formula X could not be contaminated with B.cepacia as compared with formula Y. It was recommended that the production of formula X instead of the current one is more effective to prevent the recurrence of contamination.

Fortunately, the problem of contamination in machines was resolved by applying new disinfectants but the main source of contamination remained unknown. Further investigation in Stage IV, 384 samples from water treatment station and R.O. station were tested. Of these, 35 isolates were recovered and identified. B.cepacia was overwhelmingly the most common isolates from the purified water system. This conclusion was in agreement with about the most common source of contamination which is water and aqueous products are at very high risk due to the ability of Burkholderia cepacia to remain viable in stressful and hard conditions.

Water is widely used in pharmaceutical manufacturing either as a raw material, as an ingredient, or as a final product. Water is also used for rinsing equipment or for the preparation of disinfectants and detergents. These applications require pharmaceutical grade water to be used, which is water that has been through a chemical purification step. Purified water is high grade water produced by Reverse Osmosis (R.O.). R.O. units use a semi permeable membrane to achieve microbial reductions. R.O. results by applying pressure to the concentrated side of the membrane. This pushes purified water into the dilute side. The rejected microorganisms from the concentrated side are then rinsed away.

The authors proposed different protocols aiming to control and to prevent the contamination. These treatments stated in Stage IV are Ultraviolet (UV) disinfection, chemical washing with Ethylene di-amine tetra-acetic acid (EDTA), acidic and basic wash, thermal sterilization at 110 °C for 90 minutes, Ozone sterilization, hydrogen peroxide sanitization and chlorine disinfection.

UV radiation is inappropriate for water sanitization due to the presence of soluble organic materials that can react with or absorb the UV radiation, leading to the reduction of the disinfection performance. Practically, the applying of UV radiation has no effect on contamination. Chemical washing and thermal sterilization have very short effect on contamination, so, another water disinfection method should be applied such as: ozone sterilization used every week for R.O. loop feeding syrup production line.

Ozone is the strongest oxidation agent in water treatment and purification and reuse has attracted great interest and is adding new aspects in ozonation-by product research. Studies showed the disinfection by ozone effectively kills pathogenic microorganisms such as Pseudomonas aeruginosa, Staphylococcus aureus and Burkholderia cepacia including bacteria growing in biofilms. Ozone is generally effective as a disinfectant but the results vary according to different factors such as: differences in the concentration of ozone, time of exposure along with different bacteria and growth conditions. The authors found that after 6 months of ozone disinfection, a recurrence of contamination was revealed. It is also an expensive disinfection technology operating costs and to date it has been used as a pre-disinfection treatment process for the destruction of organic micropollutants. Ozone is not stable so can’t be produced and transported to the point of use. It must be generated at the point of use. A secondary disinfectant is required.

Other method for water disinfection was used which recommended by, is the use of oxidizing disinfectants, this group includes oxygen-releasing components (peroxogens) like hydrogen peroxide (H$_2$O$_2$). They disturb the cell wall permeability, causing cytoplasm leakage and denature bacterial cell enzymes through oxidation. Oxidizing agents have advantages in that they clear and colorless, so they cannot stain the surface.

Hydrogen peroxide 3% has been used for water disinfection and showed a strong bactericidal effect against B.cepacia. The most common form of disinfection is chlorination, although ozone and UV light are also used. Chlorine continues to be one of the most effective disinfectant, it is relatively easy to handle, the costs of chlorine installation

Fig. 1:
Table 1: Bacterial samples obtained from aqueous finished products

<table>
<thead>
<tr>
<th>Code</th>
<th>on OFPBL</th>
<th>on BCSA</th>
<th>Gram stain</th>
</tr>
</thead>
<tbody>
<tr>
<td>1F</td>
<td>yellow colonies</td>
<td>yellow colonies surrounded by pink zones</td>
<td>G-ve bacilli</td>
</tr>
<tr>
<td>2F</td>
<td>yellow colonies</td>
<td>yellow colonies surrounded by pink zones</td>
<td>G-ve bacilli</td>
</tr>
<tr>
<td>3F</td>
<td>yellow colonies</td>
<td>yellow colonies surrounded by pink zones</td>
<td>G-ve bacilli</td>
</tr>
<tr>
<td>4F</td>
<td>yellow colonies</td>
<td>yellow colonies surrounded by pink zones</td>
<td>G-ve bacilli</td>
</tr>
<tr>
<td>5F</td>
<td>yellow colonies</td>
<td>yellow colonies surrounded by pink zones</td>
<td>G-ve bacilli</td>
</tr>
<tr>
<td>6F</td>
<td>blue colonies</td>
<td>white colonies</td>
<td>G-ve bacilli</td>
</tr>
<tr>
<td>7F</td>
<td>blue colonies</td>
<td>white colonies</td>
<td>G-ve bacilli</td>
</tr>
<tr>
<td>8F</td>
<td>blue colonies</td>
<td>transparent colonies</td>
<td>G-ve bacilli</td>
</tr>
<tr>
<td>9F</td>
<td>blue colonies</td>
<td>transparent colonies</td>
<td>G-ve bacilli</td>
</tr>
<tr>
<td>10F</td>
<td>blue colonies</td>
<td>white colonies</td>
<td>G-ve bacilli</td>
</tr>
<tr>
<td>11F</td>
<td>blue colonies</td>
<td>transparent colonies</td>
<td>G-ve bacilli</td>
</tr>
<tr>
<td>12F</td>
<td>blue colonies</td>
<td>transparent colonies surrounded by pink zones</td>
<td>G-ve bacilli</td>
</tr>
<tr>
<td>13F</td>
<td>blue colonies</td>
<td>yellow colonies</td>
<td>G-ve bacilli</td>
</tr>
<tr>
<td>14F</td>
<td>blue colonies</td>
<td>yellow colonies</td>
<td>G-ve bacilli</td>
</tr>
<tr>
<td>15F</td>
<td>yellow colonies</td>
<td>yellow colonies surrounded by pink zones</td>
<td>G-ve bacilli</td>
</tr>
<tr>
<td>16F</td>
<td>yellow colonies</td>
<td>yellow colonies surrounded by pink zones</td>
<td>G-ve bacilli</td>
</tr>
<tr>
<td>17F</td>
<td>yellow colonies</td>
<td>yellow colonies surrounded by pink zones</td>
<td>G-ve bacilli</td>
</tr>
<tr>
<td>18F</td>
<td>yellow colonies</td>
<td>yellow colonies surrounded by pink zones</td>
<td>G-ve bacilli</td>
</tr>
<tr>
<td>19F</td>
<td>yellow colonies</td>
<td>yellow colonies surrounded by pink zones</td>
<td>G-ve bacilli</td>
</tr>
<tr>
<td>20F</td>
<td>No Growth</td>
<td>No Growth</td>
<td>-</td>
</tr>
<tr>
<td>21F</td>
<td>No Growth</td>
<td>No Growth</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 2: Disinfectant Tego against B. cepacia after contact time 15 minutes on 4 different surfaces (Ceramic, Epoxy, Stainless-steel and glass).

<table>
<thead>
<tr>
<th>Organism</th>
<th>Positive control (CFU)</th>
<th>Recovery (CFU)</th>
<th>No. of log reductions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burkholderia cepacia</td>
<td>8x10^8</td>
<td>5.6x10^4</td>
<td>4.15</td>
</tr>
</tbody>
</table>

Table 3: Disinfectant Viragri against B. cepacia after contact time 15 minutes on 4 different surfaces.

<table>
<thead>
<tr>
<th>Surface</th>
<th>Organism</th>
<th>+ve control (CFU)</th>
<th>Recovery (CFU)</th>
<th>No. of log reductions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceramic</td>
<td>Burkholderia cepacia</td>
<td>8.4x10^8</td>
<td>7.5x10^4</td>
<td>4</td>
</tr>
<tr>
<td>Epoxy</td>
<td></td>
<td>2.5x10^4</td>
<td>2.5x10^4</td>
<td>4.5</td>
</tr>
<tr>
<td>Glass</td>
<td></td>
<td>5x10^4</td>
<td>5x10^4</td>
<td>4.2</td>
</tr>
<tr>
<td>Stainless-steel</td>
<td></td>
<td>6x10^4</td>
<td>6x10^4</td>
<td>4.1</td>
</tr>
</tbody>
</table>

Table 4: Effect of different concentrations of cremophore with parabens on B. cepacia

<table>
<thead>
<tr>
<th>Cremophore concentrations</th>
<th>1%</th>
<th>0.75%</th>
<th>0.50%</th>
<th>0.25%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl paraben</td>
<td>0.10%</td>
<td>0.10%</td>
<td>0.10%</td>
<td>0.10%</td>
</tr>
<tr>
<td>Propyl paraben</td>
<td>0.02%</td>
<td>0.02%</td>
<td>0.02%</td>
<td>0.02%</td>
</tr>
</tbody>
</table>

are low, simple to dose, measure and control and it has a relatively good residual effect. Chlorine is the most common form of water treatment used worldwide. Chlorine is widely available and can be applied in many forms and ways.  

After all these previous studies, our recommendation is the use of chlorine and hydrogen peroxide in water disinfection is the perfect method for its sterilization, while the ozone disinfection can be used only on surface machines.

At the end of Stage IV, 100% of the finished products shown in Stage IV were clear, accepted, free from any contaminant especially Burkholderia cepacia and returned to the markets safely.

Figure 1 Antimicrobial preservative efficacy results showed in form of difference in log reductions between modified formula from the susceptible odd product formula X: 0.25% cremophore, no ethanol with parabens, formula Y: 5% ethanol with parabens and the current formula 1% cremophore with parabens at the applicable test intervals (zero day, after 14 days and after 28 days).

The log reduction is defined as the difference between the log10unit value of the starting concentration of CFU/ml in the suspension and the log10 unit value of CFU/ml of the survivors at that time point.
The log reduction calculated as:

\[ \text{Log reduction} = \log_{10} \left( \frac{\text{Total Positive Control count}}{\text{Total Test Sample Count}} \right) \]

The results of these experiments showed that the formula X could not be contaminated with Burkholderia cepacia as compared with formula Y.

5. Conclusion

The characterization of the isolated organism was performed using various morphological, biochemical and physiological parameters and the result was Burkholderia cepacia group. Molecular characterization based on 16S rRNA and NCBI BLAST search confirms the identity of the test organism Burkholderia cepacia complex.

Conclusively, it is recommended to prevent the contamination of the finished products during the process of manufacturing by:

- The usage of different disinfectants which have strong effect on B.cepacia and a rotation plan between them is required.
- The pharmaceutical water stations contaminated with this microorganism must undergo treatment by using 3% hydrogen peroxide and by chlorine disinfection up to 1ppm.

6. Source of Funding

None.

7. Conflict of Interest

None.

References


Author biography

Marina Ramsis Fahmy Microbiologist

Youssry El-Sayed Saleh Professor

Mary Sobhy Khalil Professor