Microbiological Safety of Bottled Mineral Water in Patients Susceptible to Infections

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We evaluated the microbiological safety of bottled mineral water products commercially available in Japan. Of 10 bottled mineral water products manufactured in Japan, no bacteria or fungi were detected in 9 (90%), but 1 (10%) contained $1.8 \times 10^3$ colony-forming units (cfu)/mL. Of 12 bottled mineral water products manufactured in the EU, 11 (91.7%) contained $23-3.5 \times 10^4$ cfu/mL. On the other hand, of 5 bottled mineral water products manufactured in North America, 2 (40%) contained $2.3 \times 10^2-2.5 \times 10^3$ cfu/mL. The detected microorganisms were glucose-nonfermentative Gram-negative bacilli such as *Brevundimonas vesicularis*, *Moraxella* spp., and *Burkholderia cepacia*, but *Pseudomonas aeruginosa* was not detected in any product. For immunocompromised host patients being managed in ultra-clean rooms, the examined bottled mineral water products manufactured in Japan, except 1, were microbiologically safe.

Key words: mineral water; *Brevundimonas vesicularis*; *Moraxella* spp.; *Burkholderia cepacia*; immunocompromised hosts

Introduction

In recent years, the demand for bottled mineral water has been rapidly increasing. Demand is also high in the case of inpatients in hospitals. However, some bottled mineral water products contain microorganisms (1–6) that can be sources of infection for immunocompromised hosts, such as patients who have undergone hematopoietic cell transplantation and are being managed in ultra-clean rooms. To identify bottled mineral water products that are microbiologically safe for immunocompromised hosts, we evaluated microorganisms in bottled mineral water products commercially available in Japan.

Materials and Methods

Bottled mineral water

We evaluated microorganisms in 27 bottled mineral water products (10 products manufactured in Japan, 12 manufactured in the EU, and 5 manufactured in North America) purchased in retail stores in Yamaguchi Prefecture, Japan between September 20 and December 20, 2006. Three bottles differing in the expiry date or lot No. were examined for each product, excluding GLACIER and OXY GIZER. Only 1 bottle of GLACIER and 2 bottles of OXY GIZER were examined since 3 bottles differing in the expiry date or lot No. could not be purchased.

Microbial detection of bottled mineral water products

Each of the bottled mineral water products was diluted 10-fold, 100-fold, and 1000-fold in sterile saline. Subsequently, two aliquots (0.5 mL each) of each dilution and of an undiluted sample were plated onto two trypticase soy agar plates, two NAC agar plates, two salt egg yolk agar plates, or two Sabouraud dextrose agar plates. Plates were streaked with a glass “hockey stick” and incubated at 30°C for 24–72 hours (trypticase soy and NAC agar), at 35°C for 48 hours (salt egg yolk agar), and at 25°C for 2–7 days (Sabouraud dextrose agar). Colonies were counted and the organisms identified by Gram-staining, morphology, the OF test, cytochrome oxidase test, coagulase test, and the ‘API’ system.

Results

Table 1 shows the country of origin, bacterial counts/ml, and detected microorganisms for the 27 bottled mineral water products. Of 10 bottled mineral water products manufactured in Japan, no bacteria or fungi were detected in 9, but 1 contained $1.8 \times 10^3$ colony-forming units (cfu)/mL. Of 12 bottled mineral water products manufactured in the EU, 11 contained $23-3.5 \times 10^4$ cfu/mL. On the other hand, of 5 bottled mineral water products manufactured in North America, 2 contained $2.3 \times 10^2-2.5 \times 10^3$ cfu/mL. The detected microorganisms were glucose-nonfermentative Gram-negative bacilli such as *Brevundimonas vesicularis*, *Moraxella* spp., and *Burkholderia cepacia*. *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and fungi were not detected in any of the 27 products.
Our survey showed that all the bottled mineral water products manufactured in Japan, except 1, were micro-
bacteriologically safe for immunocompromised host pa-
tients. The absence of microorganisms in most ex-
amined products manufactured in Japan may be be-
cause the Food Sanitation Law require manufacturers to treat bottled mineral water for the elimination of 
microorganisms, such as by disinfection by heating at 
85°C for 30 minutes, or sterilization by filtration through a 0.45 μm membrane filter. Only one product manufactured in Japan contained microorganisms (1.8 × 10³ cfu/mL), and did not comply with the Food Sani-
tation Law regulation.

Microorganisms were detected in most of the ex-
amined products manufactured in the EU. This may be 
because natural mineral water defined in the EU is a 
product not treated except for the separation of unsta-
able elements such as iron and sulphur compounds (Eu-
ropean Community Legislation, 1980)[1]. Since bottled 
mineral water products manufactured in the EU are not 
treated for the elimination of microorganisms by dis-
fection or sterilization, some microbial contamination is expected. However, since the detection level was often 
10³–10⁴ cfu/mL, the use of bottled mineral water prod-
ucts manufactured in the EU is microbiologically in-
appropriate for immunocompromised host patients, such as patients who have undergone hematopoietic cell transplantation in ultra-clean rooms[7–11]. On the other hand, heterotrophic bacteria present in bottled mineral water have been reported to decompose formal-
dehyde and acetaldehyde migrating from the polyethy-
lene terephthalate (PET) materials of the bottle[12].

Therefore, for healthy people, bottled mineral water products manufactured in the EU do not pose a health risk.

Of the 5 bottled mineral water products manufactured in North America, no bacteria of fungi were 
detected in 3. This may be because some natural mineral 
water products are treated for the elimination of 
microorganisms by UV radiation or ozonation in North 
America[6].

Glucose-nonfermentative Gram-negative bacilli such as Pseudomonas fluorescens and Sphingomonas pauci-
mobilis detected in bottled mineral water can be sources 
of infection for immunocompromised host patients[13, 14]. Oral intake of these microorganisms imposes the risk of 
colonization of the intestine, followed by infection. Therefore, when bottled mineral water is used as drinking 
water for immunocompromised host patients, products manufactured in Japan in compliance with its Food

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### Table 1. Culture results from bottled mineral water

<table>
<thead>
<tr>
<th>Products</th>
<th>Bacterial counts/mL</th>
<th>Detected microorganisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Japanese product A</td>
<td>1.8 × 10³</td>
<td>Moraxella spp.</td>
</tr>
<tr>
<td>Japanese product B</td>
<td>ND⁵</td>
<td></td>
</tr>
<tr>
<td>Japanese product C</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Japanese product D</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Japanese product E</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Japanese product F</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Japanese product G</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Japanese product H</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Japanese product I</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Japanese product J</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>French product A</td>
<td>3.5 × 10⁴</td>
<td>Brevundimonas vesicularis</td>
</tr>
<tr>
<td>Italian product A</td>
<td>1.5 × 10⁴</td>
<td>Brevundimonas vesicularis</td>
</tr>
<tr>
<td>Italian product B</td>
<td>1.0 × 10⁴</td>
<td>Moraxella spp.</td>
</tr>
<tr>
<td>French product B</td>
<td>4.4 × 10³</td>
<td>Burkholderia cepacia</td>
</tr>
<tr>
<td>French product C</td>
<td>4.2 × 10³</td>
<td>Comamonas acidovorans</td>
</tr>
<tr>
<td>French product D</td>
<td>1.5 × 10³</td>
<td>Sphingomonas paucimobilis</td>
</tr>
<tr>
<td>French product F</td>
<td>1.3 × 10³</td>
<td>Methyllobacterium mesophilicum</td>
</tr>
<tr>
<td>British product A</td>
<td>9.3 × 10²</td>
<td>Alcaligenes faecalis, Pseudomonas fluorescens</td>
</tr>
<tr>
<td>Spanish product A</td>
<td>3.6 × 10²</td>
<td>Brevundimonas vesicularis</td>
</tr>
<tr>
<td>Italian product C</td>
<td>46</td>
<td>Pastutella spp.</td>
</tr>
<tr>
<td>French product G</td>
<td>23</td>
<td>Bacillus spp.</td>
</tr>
<tr>
<td>Italian product D</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Canadian product A</td>
<td>2.5 × 10³</td>
<td>Flavimona orisibilitas</td>
</tr>
<tr>
<td>Canadian product B</td>
<td>2.3 × 10²</td>
<td>Oligella ureolytica</td>
</tr>
<tr>
<td>Canadian product C</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>US product A</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Canadian product D</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

⁵ The mean value from a total of 3 bottles differing in the expiry date or lot No. is shown for each product, excluding Italian product D and Canadian product D. The mean value from 2 bottles differing in lot No. is shown for Italian product D, and the value from 1 bottle is shown for Canadian product D.

⁶ ND = not detected
Sanitation Law are recommended.

References


