Monitoring the Quality of Mineral Bottled Water Concerning to Potential Pathogenic Bacteria and Nitrate Levels

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Abstract—The diversity of cultured pathogenic bacteria in the bottled mineral water (BMW) was investigated using selective media. The pure isolates from these selective media, which showed hemolytic activity on the blood agar media and antibiotic resistance, were identified by 16S rRNA gene technique. The seven obtained strains were belonged to the genus Pseudomonas, Bacillus, Acinetobacter, Stenotrophomonas, and Exiguobacterium, and were mostly closed to the pathogenic strains. The increasing of ozone concentration from air-fed ozone generators eliminate the growth of bacteria included the pathogenic bacteria, but in other side it increases the amount of nitrates and nitrates in the final product of the BMW. These findings revealed that the BMW either has potential pathogenic bacteria or high levels of nitrates and all these products may effect on the health of the end user.

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Keywords: bottled mineral water, pathogenic bacteria, 16S rRNA gene sequence, ozone and nitrates.

INTRODUCTION

Bottled mineral water (BMW) as an oligotrophic environment should have viable bacterial cell content as low as 10 cfu ml⁻¹ [1–2]. These lows, count of native organisms are of little concern to the healthy consumer. Bacterial communities of BMW and tap water originated from the same sources may contain the same bacterial communities [3]. Waterborne pathogens may infect 350 million people within those people 10–20 million succumbing to severe cases [4]. This phenomenon is far from being restricted to developing countries but also threatens developed countries. From 1991 to 1999 in the USA 430,000 cases were infected by 126 waterborne infectious diseases outbreaks [5]. BMW represent one of the largest sectors by volume in the Egyptian soft drinks market [6]. The treatments for producing bottled water processed by sedimentation, sand filtration, carbon filtration, green sand filtration, microfiltration, ultraviolet disinfection and ozone disinfection. Bacteria enter the potable water as a result of its attachment to carbon fines in carbon filter [7]. Inducing of BMW contamination may be from the bacteria and impurities which clog the filter or from potential pathogen bacteria remain in the underground mineral water or during the bottling process itself [8]. Because there is no effective sterilization process of commercially mineral waters for removal of all microorganisms, where the pathogen may be having a way to cause infection for end user. Several bacterial species contaminated the bottled drinking water as: E. coli, Salmonella typhimurium, Pseudomonas aeruginosa, Campylobacter jejuni and Aeromonas hydrophila [9]. In Germany hospital the intensive care units infected with P. aeruginosa as a result of contamination BMW used for the preparation of orally administered medications and oral fluid replacement [10]. Also Salmonella sp. was isolated from BMW from the markets by randomly selection and in the local BMW factory [11]. In 1974, there was an outbreak of cholera by Vibrio cholera associated with bottled mineral waters in Portugal, 2467 bacteriological confirmed cases, 48 were dying, while 82 patients had a history of drinking bottled mineral water from one particular source other than bottled water, this means that bottled water was not the only cause of the outbreak. Vibrio cholera was found in the water source after 36 cases had visited the spa served by the same water source as the bottled water [12, 13].

Gram-positive cocci isolated from bottled mineral waters as Staphylococcus micrococcii, S. yours, S. epidermidis, S. hominis and S. wameri were identified [9]. The autochthonous flora of bottled water is a complex ecosystem with great heterogeneity; they are generally psychrophilic and oligocarbotrophic and they multiply rapidly in the bottled water as (Acinetobacter, Moraxella, Aeromonas, Xanthomonas etc.). The autochthonous flora has the potential for the causing diseases are not clear. Aeromonas is sometimes associated with wound infec-

1 The text was submitted by the authors in English.
tions and suspected to be a causative agent of diarrhea. Several *Pseudomonas sp.* can cause disease in humans. *P. cepacia* is increasingly identified as a cause of serious chest infections in children with cystic fibrosis. *Acinetobacter sp.* can be a problem on intensive care units. *Mycobacterium sp.* may be a cause of pulmonary disease. *Moraxella* can cause infections of the eye and upper respiratory tract. Spreads of waterborne infectious diseases via the use of BMW even in low count still pose a serious health threat worldwide.

BMW consumers are spread all over Egypt, and any waterborne infectious diseases will be difficult to control. In this study, not only potential bacterial pathogens were screened but also chemical and physical conditions were examined to understand the environmental condition for these pathogens, as well as the effect of ozone treatment on BMW and nitrogen oxide formation.

**EXPERIMENTAL**

**Water Samples**

Fifty samples were collected from treatment processing units of BMW and also from the final product (BMW) from 8 factories for BMW in Wadi El Natroon region, where most of the Egyptian BMW manufactory are in Wadi El Natroon region. Samples were collected aseptically from several treatments processing units as: sand filter, carbon filter, green sand filter, 1 micro filter 0.45 micro filter, storage tanks, ozone disinfection towers and final product. The collected water samples were filtered from bacteria using a thin filter 0.45 μm (Sartorius stadium Biotech). The retained bacteria on the filter were grown on selective media: mannitol salt agar, Endo agar base, Difco Pseudomonas isolation agar and Clostridium agar [14], each trapped bacterium on the filter was grown into a colony.

**Monitoring Physical and Chemical Conditions of the Water Samples**

Physical conditions as pH, temperature and turbidity were measured, while the chemical conditions as total hardness, calcium, magnesium, bicarbonate, potassium, chloride, sulphate, total dissolved salts (TDS), ammonia, nitrates, nitrite, silica, manganese and iron were detected, all the chemical analysis were determined by the Procedures recommended in the standard methods for the examination of water and wastewater [15], nitrate levels were detected by DR 2800 Spectrophotometer (HACH) with use Catalogue number: DOC022.53.00725 and its chemical reagent.

**Growth on Blood Nutrient Agar Media**

The pure colonies on the filter membrane grown on selective media: mannitol salt agar, endo agar base, Difco Pseudomonas isolation agar and Clostridium agar were purified on nutrient agar media, and cultured on blood agar plates and incubated at 37°C for 24 h. The observation of clear zones around the bacterial colonies showed β-haemolysis, whereas green zones around the colonies suggested α-haemolysis and no haemolysis was called γ-haemolysis.

**Antibiotic Susceptibility Tests for Potentially Pathogenic Heterotrophic Bacteria**

The antibiotic susceptibility of the bacterial isolates were tested using the disk diffusion method. Ten types of antibiotic disks were placed on the inoculated plates with sterile forceps antibiotics were purchased from bioanalyse; these antibiotics were: ceftriaxone (30 μg), ampicillin (10 μg), cefprozil (30 μg), ofloxacin (5 μg), amikacin (30 μg), ciprofloxacin (5 μg), amoxicillin/clavulanic acid (20 μg/10 μg), chloraphenicol (30 μg), cloxacillin (1 μg) and rifamycin (30 μg).

**Phylogenetic Analysis**

Genomic DNA of the pure isolates were extracted using DNA purification GeneJET™ Genomic DNA Purification kit (Thermo Scientific), using gram-positive bacteria genomic DNA purification protocol. Three primers were used in the amplification of 16S rRNA. These include: Bact27f (5’- AGAGTTTGATC (A/C)-TGGCTCAG-3’), Bact1492r (5’-TACGG(C/T)TACCTTGTTACGACTT-3’), and Bact1098r (5’-AAGGGTTGCGCTCGTGGG-3’)[16]. Theoretically, amplification with Bact27f -1492r should yield 1505bp and amplification with Bact27f -1098r should yield 1108bp from the 16S rRNA. PCR amplification was performed in a total volume of 50 μl in model T Personal thermocycler (Biometra). Each PCR mixture contained 25 ng of template DNA, 0.6 μM of each primer, 1.75 mM MgCl₂, 200 μM of dNTPs, 1.25 U of Taq