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**CASE REPORT**

**PATHOLOGY/BIOLOGY**

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# Characterization of Microorganisms Isolated from Counterfeit Toothpaste

**ABSTRACT:** The appearance of potentially counterfeit "Colgate" toothpaste on the American market prompted a criminal investigation by the United States Food and Drug Administration (FDA), including the collection of *c.* 60,000 tubes of toothpaste from retail outlets and product distributors. Microbiological testing was performed based on the FDA Bacteriological Analytical Manual, which determined the presence and number of bacteria present in the products. Bacteria were isolated from each "Colgate" variety; up to $2 \times 10^6$ cfu/g were isolated from some of the product units. Using conventional microscopic and biochemical bacterial identification methods, most of the bacteria isolated from these samples were Gram-negative rods of several genera, including *Pseudomonas*, *Serratia,* and *Klebsiella.* Most of the organisms isolated represent opportunistic pathogens, and therefore, counterfeit "Colgate" toothpaste containing high levels of bacteria pose a human health hazard.

**KEYWORDS:** forensic science, toothpaste, counterfeit drug, bacteria, Colgate, enterobacteriaceae

In the spring of 2007, United States Immigration Customs Enforcement agents seized over 144,000 tubes of allegedly counterfeit "Colgate" toothpaste in Newark, NJ (1). Subsequently, the Food and Drug Administration (FDA) Office of Criminal Investigations began an investigation that culminated in the collection of 60,000 additional tubes of counterfeit toothpaste, primarily from distributors and discount retailers on the East coast. All of the counterfeit products bore the "Colgate" name and included several product types, including "Colgate Herbal Gel," "Colgate Triple Action," "Colgate Gel," and "Colgate Maximum Cavity Protection." Although the product labels claimed that they were manufactured in South Africa, the actual country of origin was China. Eventually, these products were found in multiple U.S. states, as well as world-wide distribution in places as diverse as the Cayman Islands, Nigeria, Spain, and Canada (2–5). Studies from both Europe and Canada demonstrated the presence of high numbers of bacteria in these products (2,5). The following study describes the isolation and characterization of the bacterial species from counterfeit Colgate products collected in the United States.

## Materials and Methods

### *Microbiology*

The FDA Bacteriological Analytical Manual (6) method for cosmetics was used to determine the approximate bacterial load of each sample. Briefly, 1 g of each unit was serially diluted in 9 mL of modified Letheen Broth (MLB; General Laboratory Products, Bolingbrook, IL). One hundred microliters of selected dilutions ($10^{-1}$, $10^{-3}$, and $10^{-5}$) were plated to duplicate Modified Letheen Agar plates (MLA; Biomerieux, Hazelwood, MO) incubated for 48 h at 30°C and enumerated. Each unique bacterial isolate, based on colonial morphology, was identified and streaked for isolation onto trypticase soy broth agar containing 5% sheep's blood (SBA). Each isolate was subsequently Gram-stained and identified using the Vitek 2 Compact identification system (Biomerieux, Hazelwood, MO). The MLB dilutions were enriched for growth for up to 7 days at 30°C after direct plating. If the direct plates were negative for bacterial growth 48 h following initial plating, enrichments were plated onto MLA following 7 days of incubation or when turbidity was detected. If bacteria were isolated from the enrichments plated onto MLA, they were cultured onto SBA, characterized, and identified in the same manner as the isolates from the direct plate method.

Bacterial identification based on 16S rDNA sequencing was performed on isolates by the Centers for Disease Control and Prevention (CDC), Atlanta, GA.

## Results and Discussion

Bacteria were isolated from 38 of the 63 samples analyzed (60%). Bacterial species identified during the analysis were gram-negative rods and gram-positive bacilli; no gram-positive cocci were isolated from any of the products. Table 1 shows the composition of each product unit analyzed, grouped by product type. The FDA guideline for an acceptable amount of bacteria in a cosmetic is $1 \times 10^3$ cfu/g (6). Sixteen of the 17 tubes of "Colgate Herbal" analyzed contained significant bacterial contamination, ranging from *c.* $2.5 \times 10^4$ to $2.5 \times 10^6$ organisms/g. Of the 16 tubes of "Colgate Triple Action" analyzed, 10 were found to be contaminated with bacteria. Thirteen tubes of "Colgate Gel" were tested, and six of them were found to contain bacteria. Of the 17 tubes of "Colgate Maximum Cavity Protection" analyzed, bacteria were isolated from six units.

As shown in Table 1, most of the products examined were harboring only one or two species of bacteria. Members of the genus *Pseudomonas* were isolated from three of the four product types analyzed. Indeed, the predominant contaminants were

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TABLE 1—*Microbiological results by product name.*

| ID | Product Name | Cfu/g[†] | Organisms Isolated |
|---|---|---|---|
| H1 | "Herbal" | $6.1 \times 10^4$ | *Pseudomonas stutzeri; Acinetobacter haemolyticus* |
| H2 | "Herbal" | $5.2 \times 10^4$ | *P. stutzeri; A. haemolyticus* |
| H3 | "Herbal" | $2.2 \times 10^5$ | *Pseudomonas putida* |
| H4 | "Herbal" | Negative | |
| H5 | "Herbal" | $1.8 \times 10^5$ | *P. putida* |
| H6 | "Herbal" | $3.3 \times 10^5$ | *Pseudomonas sp. (mendocina or putida)* |
| H7 | "Herbal" | $2.5 \times 10^6$ | *Enterbacter gergoviae; Pseudomonas aeruginosa* |
| H8 | "Herbal" | $3.9 \times 10^5$ | *E. gergoviae* |
| H9 | "Herbal" | $5.2 \times 10^4$ | *P. putida* |
| H10 | "Herbal" | $4.8 \times 10^4$ | Unidentified |
| H11 | "Herbal" | $5.2 \times 10^4$ | *Serratia plymuthica;* Unidentified |
| H12 | "Herbal" | $6.2 \times 10^5$ | *P. putida* |
| H13 | "Herbal" | $4.9 \times 10^5$ | *P. putida* |
| H14 | "Herbal" | $2.7 \times 10^6$ | *S. plymuthica* |
| H15 | "Herbal" | $2.7 \times 10^4$ | *Brevibacillus choshinensis; Aeromonas salmonicida;* two Unidentified |
| H16 | "Herbal" | $8 \times 10^5$ | *Unidentified* |
| H17 | "Herbal" | $2.3 \times 10^5$ | *Unidentified* |
| T1 | "Triple Action" | Negative | |
| T2 | "Triple Action" | Negative | |
| T3 | "Triple Action" | $2.6 \times 10^5$ | *Klebsiella oxytoca; P. stutzeri* |
| T4 | "Triple Action" | <500 | *Bacillus megaterium; S. plymuthica* |
| T5 | "Triple Action" | Enrichment[*] | Unidentified |
| T6 | "Triple Action" | Enrichment[*] | Unidentified |
| T7 | "Triple Action" | $1.4 \times 10^5$ | *P. stutzeri;* Unidentified |
| T8 | "Triple Action" | $3.4 \times 10^5$ | *P. stutzeri* |
| T9 | "Triple Action" | Negative | |
| T10 | "Triple Action" | Negative | |
| T11 | "Triple Action" | $1.8 \times 10^5$ | *K. oxytoca; P. stutzeri* |
| T12 | "Triple Action" | $3.9 \times 10^5$ | *K. oxytoca; P. stutzeri, A. salmonicida* |
| T13 | "Triple Action" | Negative | |
| T14 | "Triple Action" | Negative | |
| T15 | "Triple Action" | $1.2 \times 10^6$ | *P. stutzeri;* Unidentified |
| T16 | "Triple Action" | $2.1 \times 10^5$ | *P. stutzeri;* Unidentified |
| G1 | "Fluoride Gel" | Enrichment[*] | *B. megaterium* |
| G2 | "Fluoride Gel" | Negative | |
| G3 | "Fluoride Gel" | $1.9 \times 10^6$ | *Halotalea alkalilenta* |
| G4 | "Fluoride Gel" | Negative | |
| G5 | "Fluoride Gel" | Negative | |
| G6 | "Fluoride Gel" | Negative | |
| G7 | "Fluoride Gel" | Negative | |
| G8 | "Fluoride Gel" | $1.7 \times 10^4$ | *H. alkalilenta* |
| G9 | "Fluoride Gel" | $3.1 \times 10^4$ | *A. haemolyticus; H. alkalilenta* |
| G10 | "Fluoride Gel" | Negative | |
| G11 | "Fluoride Gel" | Negative | |
| G12 | "Fluoride Gel" | Enrichment[*] | *Bacillus pumilus* |
| G13 | "Fluoride Gel" | Enrichment[*] | *S. plymuthica* |
| M1 | "Maximum Cavity Protection" | Negative | |
| M2 | "Maximum Cavity Protection" | Negative | |
| M3 | "Maximum Cavity Protection" | Enrichment[*] | *Burkholderia cepacia* group; Unidentified |
| M4 | "Maximum Cavity Protection" | Negative | |
| M5 | "Maximum Cavity Protection" | Negative | |
| M6 | "Maximum Cavity Protection" | $9.2 \times 10^5$ | *Citrobacter koseri; Pantoea sp.* |
| M7 | "Maximum Cavity Protection" | Negative | |
| M8 | "Maximum Cavity Protection" | Enrichment[*] | Unidentified |
| M9 | "Maximum Cavity Protection" | Enrichment[*] | *Bacillus sp. (thuringensis, mycoides, or cereus)* |
| M10 | "Maximum Cavity Protection" | Enrichment[*] | *Pseudomonas luteola;* Unidentified |
| M11 | "Maximum Cavity Protection" | Enrichment[*] | *P. luteola;* Unidentified |
| M12 | "Maximum Cavity Protection" | Negative | |
| M13 | "Maximum Cavity Protection" | Negative | |
| M14 | "Maximum Cavity Protection" | Negative | |
| M15 | "Maximum Cavity Protection" | Negative | |
| M16 | "Maximum Cavity Protection" | Negative | |
| M17 | "Maximum Cavity Protection" | Negative | |

[*]Isolates were derived only from liquid enrichments; direct plating analyses were negative in these cases.
[†]Colony-forming units/g of product.

Pseudomonads, comprised of four species of the genus *Pseudomonas* and the related genus, *Acinetobacter*. These bacteria are widely distributed in nature, especially in soil and water (7). Some members of the genus can be commensal organisms, living in the human oral cavity, but others, such as *Pseudomonas aeruginosa*, are commonly associated with human infection (8).

Another family of bacteria that was identified in the analysis was the *Enterobacteriaceae*. This is a large family of

gram-negative rods, most of which are normally present in the mammalian intestinal tract (9). Members of this bacterial family were isolated from all of the products analyzed, namely members of the genera *Enterobacter*, *Klebsiella*, *Citrobacter*, *Panotea,* and *Serratia*. *Serratia plymuthica* was isolated from three of the four products tested; this organism can be isolated from soil and water and has been isolated from human sputum (10). Although the bacteria isolated from these products are not considered typical pathogens, they are all capable of causing infection in humans and may therefore pose a threat to individuals with weakened or compromised immune systems. All of the organisms that were not identified using the Vitek 2 were also gram-negative rods.

Approximately half of the tubes of the "Colgate Fluoride Gel" were negative for bacterial contamination. However, in this sample group, a unique organism was found, *Halotalea alkalilenta*, a newly characterized gram-negative rod that was first isolated from the waste of an olive oil mill (11). This bacterium was initially identified as *Burkholderia pseudomallei* using the Vitek 2. The Vitek 2 identifies bacterial species based on a series of biochemical tests, the results of which are compared with an internal database. As *B. pseudomallei* is classified as a Select Agent by the CDC, DNA sequence analysis of the 16 rDNA gene was required for a confirmatory identification on these isolates. On the basis of DNA analysis, the organism submitted to the CDC was a 99.5% match to *H. alkalilenta*. It is unlikely that this recently discovered, rare organism was present in the Vitek 2 database, making an accurate identification impossible using this instrumentation. *H. alkalilenta* was not isolated from any of the other toothpaste matrices and may suggest that the various products were made in multiple locations with varying bacterial flora. The pathogenicity of this species is unknown.

Taken together, the high numbers and types of bacteria isolated from these products indicate poor hygiene at the production location(s). The manufacturing process of these products has not been elucidated; it is currently unknown how or when during production these products became contaminated. Both the boxes and tubes of most of the products analyzed were stamped with "codes." However, because the product is counterfeit, these codes lack meaning. There was no correlation between the codes and the number/type of organisms isolated from the products (data not shown), suggesting that these codes cannot be used for a typical "trace-back" analysis. As the number of manufacturing sites are unknown, it is merely speculative to suggest that all of the units of any given product type were manufactured at a single site, which may confound any effort to correlate the microbiological data with chemical composition or other factors associated with the finished product.

In conclusion, bacterial contamination of counterfeit Colgate toothpaste products was common, with as many as two million bacteria/g being isolated from some of the units. The organisms identified were generally gram-negative rods, bacteria that can live in many types of environments. Most of these organisms are opportunistic pathogens, and they pose a public health threat to individuals with compromised immune systems, including the very young, the elderly, and patients with certain medical conditions.

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