TOPS OF BEVERAGE CANS ARE A POTENTIAL SOURCE OF INFECTION: A STUDY OF BACTERIAL LOAD PRESENT ON THE LIDS OF BEVERAGE CANS

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Abstract

It is common practice to drink directly from tops of beverage cans which are exposed to environmental contaminants during handling and storage. The purpose of this research was to determine the bacterial load present on the lids of beverage cans and find out the ways to significantly reduce the number of bacteria present on them. One hundred and eighty apparently clean and non-refrigerated beverage cans were collected from different shops and divided into two groups. First group was used for cleaning experiments and second group was used to determine the effect of refrigeration on bacterial load. Different types of bacteria were isolated which belonged to Bacillus spp, Staphylococcus spp, Corynebacterium spp, Streptococcus spp, Klebsiella spp and Escherichia spp. AST result pattern of S. aureus isolates did not show the presence of MRSA (Methicillin-resistant S. aureus) and MSSA (Methicillin-susceptible S. aureus). E. coli isolates were found to be highly sensitive to ceftriaxone and highly resistant to erythromycin. Cleaning with tap water plus wiping with dry tissue resulted in maximum removal of bacterial load (76.6%) as compared to other methods (68.8% with dry tissue and 47.3% with tap water) and is thus the most effective method for this purpose. Refrigeration lowered the bacterial load by 16.6%, but it depends upon the type of bacteria present. Beverage cans are present in different environments and are handled by different people and thus can be a potential source of infection for the
consumers. It is highly recommended that beverage cans should be cleaned before drinking.

Keywords

Beverage cans, Bacterial load, Consumer, Antibiotics, Refrigeration

INTRODUCTION

Usage of beverage cans is increasing day by day and people are in a habit of drinking directly from the cans. This can lead to serious consequences as the lid is highly contaminated with microorganisms (Fekete, 2018; Gündüz et al., 2019). The contamination of beverage cans before arriving to the customer can occur anywhere from the point of manufacturing to the point of storage in the stores. These cans are stored in the refrigerator or displayed on the shelves with lids uncovered. Thus, exposing the lids to the dirt and germs that will directly come into contact with the consumer’s mouth (Abraham et al., 2018; Dawson et al., 2018).

It is to be noted that the dirt is not the only culprit of contamination here. Beverage cans before arriving to the customer can also be contaminated from unhygienic handling, insects and rodents (Gündüz et al., 2019). Places which have drainage areas often contain moisture which attracts many insects and rodent species. Among insects, cockroaches are the most important reservoirs of infectious pathogens such as Salmonella spp that can contaminate food products. Dust can also harbor Salmonella spp which can remain viable there for up to 10 months (Michaels et al., 2003). Rats are notorious for about 35 rat-borne diseases of which some are directly transmitted from the feces, urine, saliva and bites from rats while some are indirectly transmitted through an arthropod vector. Important rat-borne disease includes bacterial zoonotic diseases such as bubonic plague commonly known as Black Plague, Leptospirosis, Salmonellosis and many viral zoonotic diseases (CDC, 2010).

No case of illness caused by drinking directly from the dirty beverage cans have been reported in Pakistan. This could be due the fact that people usually consume refrigerated beverage cans which may reduce the contamination level and also most people use straws or prefer to use glass or cup to drink beverages which prevents the direct contact with the lids of beverage cans.

The aim of this study was to determine the hygienic conditions of the lids of beverage cans, compare different cleaning methods such as wiping with dry tissue, rinsing under running tap
water and rinsing with tap water plus wiping with dry tissue, determine the antibiotic susceptibility pattern of isolated *S. aureus* and *E. coli* isolates against commercially available antibiotics.

**MATERIAL AND METHODS**

*Sample collection*

A total of 180 apparently cleaned, non-refrigerated beverage cans were collected (convenience sampling) in sterilized plastic zipper bags to avoid environmental contamination from different retail shops, departmental stores and drink corners. These cans were divided into 2 groups. First group contained 150 cans which were used for cleaning experiment and second group contained 30 cans which were used to check the effect of low temperature on bacterial load.

*Detection of bacterial diversity and antibiotic susceptibility testing*

Types of bacterial were identified in all 180 cans. Bacterial groups were identified through biochemical testing and by the scheme provided in Bergey’s Manual of Determinative Bacteriology. Antibiotic susceptibility test AST was performed on *S. aureus* and *E. coli* isolates by Kirby-Bauer disc diffusion method on Muller Hinton Agar MHA.

*Cleaning experiment*

For cleaning experiment cans of first group were subdivided into 3 groups in such a way that each subgroup contained 50 cans (labelled as dry tissue paper, tap water and tap water plus dry tissue paper). Each group was cleaned with sterilized dry tissue paper, rinsed with tap water (30 seconds) and rinsed with tap water (30 seconds) plus cleaned with dry tissue paper. The lid of each can including the mouthpiece (area where mouth makes contact with can) was divided into two halves. One half was swabbed before cleaning and whole lid was swabbed after cleaning (Figure 1a-b). It was assumed that bacteria were uniformly distributed over each lid.

*Effect of refrigeration*

To check the effect of refrigeration on bacterial load above-mentioned protocol was followed on second group cans with slight modifications (one half was swabbed before refrigeration and whole lid was swabbed after refrigeration at 4°C for 24 hours) as shown in Figure 1a and 1b.
Figure 1a: Grid area was used to take sample from the beverage cans before cleaning and refrigeration.

Figure 1b: Grid area was used to take sample from the beverage cans after cleaning and refrigeration.

Statistical analysis

Paired t-test was applied to detect the difference before and after cleaning. P-value ≤ 0.05 was taken as significant.
RESULTS

Detection of bacterial diversity

In this study apparently clean and non-refrigerated beverage cans were used. Of the total 180 beverage cans, Gram positive bacteria were found to be present on all samples (100%) while Gram negative bacteria were found only on 48 (26.6%) samples. Among identified Gram positive bacteria, *Bacillus* spp were distributed on all samples (100%), followed by *Staphylococcus* spp on 168 (93.3%) samples, *Corynebacterium* spp on 11 (6.1%) and *Streptococcus* spp on 4 (2.2%) samples. Among *Staphylococcus* spp, *S. aureus* were present on all 168 (100%) samples. Among Gram negative bacteria, *Escherichia* spp mainly *E. coli* were found on all 48 (100%) samples, followed by *Klebsiella* spp 13 (27%) and no *Salmonella* spp and *Pseudomonas* spp were detected.

Antibiotic susceptibility testing

The antibiotic susceptibility pattern of all *E. coli* isolates (N = 48) showed highest sensitivity to ceftriaxone 48 (100%), amikacin 40 (83.3%), ciprofloxacin 39 (81.2%) ampicillin 37 (77.1%), the lowest sensitivity was observed in erythromycin 19 (39.6%), trimethoprim/sulfamethoxazole 35 (72.9%) and chloramphenicol 36 (75%). The antibiotic susceptibility pattern of all *S. aureus* isolates (N = 168) showed highest sensitivity to vancomycin 168 (100%), linezolid 168 (100%) and trimethoprim/sulfamethoxazole 145 (86.3%), the lowest sensitivity was observed in erythromycin 56 (33.3%), and ciprofloxacin 60 (35.7%). Antibiotic susceptibility patterns of *S. aureus* and *E. coli* isolates are summarized in Table 1a and 1b, respectively.

Table 1a: Antibiotic susceptibility pattern of *S. aureus* isolates isolated from lids of beverage cans.
Table 1b: Antibiotic susceptibility pattern of *E. coli* isolates isolated from lids of beverage cans.

<table>
<thead>
<tr>
<th>Antibiotic Class</th>
<th>Antibiotics</th>
<th>Sensitive N (%)</th>
<th>Intermediate N (%)</th>
<th>Resistant N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aminoglycosides</td>
<td>Amikacin</td>
<td>40 (83.3%)</td>
<td>8 (16.7%)</td>
<td>0</td>
</tr>
<tr>
<td>Antifolate/Sulfonamides</td>
<td>Trimethoprim/Sulfamethoxazole</td>
<td>35 (72.9%)</td>
<td>0</td>
<td>13 (27.1%)</td>
</tr>
<tr>
<td>Cephalosporin</td>
<td>Ceftriaxone</td>
<td>48 (100%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fluoroquinolones</td>
<td>Ciprofloxacin</td>
<td>39 (81.2%)</td>
<td>9 (18.8%)</td>
<td>0</td>
</tr>
<tr>
<td>Macrolide</td>
<td>Erythromycin</td>
<td>19 (39.6%)</td>
<td>0</td>
<td>29 (60.4%)</td>
</tr>
<tr>
<td>Penicillin</td>
<td>Ampicillin</td>
<td>37 (77.1%)</td>
<td>0</td>
<td>11 (22.9%)</td>
</tr>
<tr>
<td>Phenicol</td>
<td>Chloramphenicol</td>
<td>36 (75%)</td>
<td>4 (8.3%)</td>
<td>8 (16.7%)</td>
</tr>
</tbody>
</table>

**Cleaning experiment**

Bacterial load was significantly reduced by 68.8% (*P* = 0.0002), 47.3% (*P* = 0.0005) and 76.7% (*P* = 0.0001) after cleaning with dry tissue paper, rinsing with tap water and rinsing with tap water plus cleaning with dry tissue paper respectively as shown in Table 2 and Figure 2.

**Table 2:** Comparison of different cleaning methods and effect of refrigeration on the number of bacterial colonies.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Colonies</th>
<th>Percentage Cleaned</th>
<th>P-value</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Before Cleaning</td>
<td>After Cleaning</td>
<td></td>
</tr>
<tr>
<td>Dry Tissue</td>
<td>3154</td>
<td>582</td>
<td>68.8</td>
</tr>
</tbody>
</table>

54
<table>
<thead>
<tr>
<th>Paper</th>
<th>Before Refrigeration</th>
<th>After Refrigeration</th>
<th>Percentage Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tap Water</td>
<td>2906</td>
<td>968</td>
<td>47.3</td>
</tr>
<tr>
<td>Tap Water + Dry Tissue Paper</td>
<td>2871</td>
<td>378</td>
<td>76.7</td>
</tr>
<tr>
<td>Non-refrigerated Cans</td>
<td>3139</td>
<td>2245</td>
<td>16.6</td>
</tr>
</tbody>
</table>

(A) Dry Tissue Paper

(B) Tap Water
**Effect of refrigeration**

Beverage cans were refrigerated to check the effect of low temperature on the number of bacteria present on the lids. Bacterial load was reduced by 16.6% ($P = 0.0024$) and was observed in all samples (100%) as shown in Table 1 and Figure 2.

**DISCUSSION**

In this study, apparently clean and non-refrigerated cans were collected from different shops and from different locations. All of these cans were found to be contaminated with a variety of bacterial species. Isolated bacteria belonged to *Bacillus* spp, *Staphylococcus* spp, *Corynebacterium* spp, *Streptococcus* spp, *Escherichia* spp and *Klebsiella* spp. Identified bacteria were *S. aureus* and *E. coli*. The presence of different types of contaminants indicated that the bacterial load present on the lids of cans mainly depends on the environment in which they are...
placed and also on the microbial flora of the human handling them. Similar bacteria were reported on lids of beverage cans (Michaels et al., 2003), other inanimate objects such as keyboards mobile phones (Koscova, Hurnikova, & Pistl, 2018), ICU equipments (Nazeri et al., 2019) and Automated Teller Machines ATM (Dawodu & Akanbi, 2021).

*S. aureus* is the normal flora of human and is present on objects which are frequently touched by human hands (Bhatta et al., 2018), indicated the possible human handling of the beverage cans. A study published by Domon et al. in (2015) reported that MRSA and MSSA cannot survive on inanimate dry objects such as on shopping baskets. The AST results of present study did not show the presence of MRSA and MSSA on the lids of beverage cans which are in agreement with the above-mentioned study. This indicates that *S. aureus* are likely to be transmitted from the healthy human handlers.

Presence of *E. coli* is considered the indicator of fecal contamination, suggesting the possible contamination through the unhygienic conditions of person handling them (Abraham et al., 2018). According to Centers for Disease Control and Prevention CDC depending upon the type of strain, ingestion of *E. coli* can cause wide range of symptoms including bloody diarrhea, fever and vomiting (CDC, 2021). Drinking directly from the beverage cans contaminated with *E. coli* can cause serious infection. In present study AST pattern of *E. coli* against antibiotics ceftriaxone (N = 48, 100%), amikacin (N = 40, 83.3%), ampicillin (N = 37, 77.1%), chloramphenicol (N = 36, 75%), and ciprofloxacin (N = 39, 81.2%) follow almost the same pattern reported by Fratamico, et al. in (2008), who reported the sensitivity of shiga toxin-producing *E. coli* (N = 219) isolated from swine feces against antibiotics amikacin, ceftriaxone, ampicillin, chloramphenicol, and ciprofloxacin as 100%, 99.5%, 84.9% 75.3%, and 99.5%, respectively (Fratamico et al., 2008).

In order to reduce the bacterial load, three different cleaning methods were employed. A study published by Michaels et al. (2003) showed the most effective method to remove contamination from tops of food and beverage cans is rinsing with tap water and then wiping with paper towel. Wiping with paper towel did not show any better results than rinsing with tap water and wiping with moist tissue paper (Michaels et al., 2003). The results of present study showed significant reduction of bacterial load in all three cleaning methods and are in agreement with the above-mentioned study but did not follow the same trend. Cans that were first rinsed with running tap
water and then wiped with dry tissue showed good results followed by wiping with dry tissue and rinsing with tap water. The possible explanation in present study is tissue paper which were already sterilized and packed in plastic wrappers and were opened only when required for cleaning. Dry tissue papers use friction to mechanically remove the dust and contaminants from the lids (Michaels et al., 2003), while tap water use high washing flow to mechanically detach bacteria (Uhlig et al., 2017) thus, reduced the already present bacterial load. Another explanation is that there could be a difference of bacterial populations in tap waters used in our study and the mentioned above. Bacteria are also present in water (Cabral, 2010). It is difficult to detect the exact quantity in running tap water. At one time there is no microorganism present in it but at other times it is loaded with huge quantity and variety of microorganisms. Rinsing with water in addition to removing must have added more and different bacteria on beverage cans but not to the point where the bacterial load was increased from the original value.

In countries like Pakistan where the weather is hot, refrigerated beverage cans are consumed. To check whether low temperature has any effect on the bacteria present on the lids of beverage cans bacterial load was determined before and after refrigeration. Effect of low temperature on E. coli introduced on the lids of beverage cans has been published by Dawson et al. (2018) in which they reported the decrease in E. coli counts after prolonged storage at refrigeration temperature. They also reported that E. coli counts decrease more rapidly in cans placed under room temperature compared to cans placed at low temperature due to the retention of moisture. Similar results have been reported by Wilks, Michels, and Keevil. (2005), who showed the survival of E. coli O157 on metal surfaces at different temperatures and time. In present study, refrigeration lowered the bacterial load to some extent. The possible explanation of reduction in the bacterial load after refrigeration for 24 hours is that bacteria living in the environment are challenged by different stressors (Batool, Yrjälä, & Hasnain, 2014). In the case of beverage cans these stressors are low nutrient levels and low water content. Beverage cans during refrigeration faced another stress which was low temperature or cold stress. Refrigeration favors the growth of psychrotrophs (Tatini and Kauppi, 2002). According to the Food Safety Information provided by United States Department of Agriculture Food Safety and Inspection Service USDA revised in May 2010, refrigeration slows bacterial growth. But because bacteria were already present in the stressful environment, addition of another stress (cold stress) must have killed some of the
bacteria and hence the bacterial load was reduced. But it depends upon the type of bacteria present.

CONCLUSION

Beverage cans are exposed to different environmental contaminations including handling by different people and are thus loaded with different types of microorganisms which can be a potential source of infection for the consumers. It is therefore highly recommended that lids of beverage cans should be cleaned before directly applying mouth to them for drinking.

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REFERENCES


