



EFFECTS OF SELECTED CARBONATED BEVERAGE ON CULTIVATED MICROORGANISMS FROM PUBLIC TOILET

Genetics

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ABSTRACT

This study was conducted by cultivating microbes from a public toilet in a nutrient agar plate as the primary culture. The antimicrobial effect was determined by disk plate method, a common laboratory procedure. In our research work, Minimum Inhibitory Concentration was determined by using different media ratios and the selected beverage-Coke on uniform amounts of microbial inoculum. At the end of the study, it was found that even though coke is used to clean the toilet according to the latest social media information like Instagram, it does not act as an antimicrobial agent as no zone of inhibition was seen around the colonies but on the contrary, a much heavier growth was observed in areas around coke-infused disks due to high sugar content acting as a source of nutrient for the growth of microorganisms.

KEYWORDS

Carbonated beverage-coke, antimicrobial effect, Minimum Inhibitory Concentration, disk plate method.

INTRODUCTION:

Carbonated beverages, also called soft drinks, are among the most widely consumed refreshment beverages worldwide. One such carbonated beverage used in our research work is Coke also known as Coca-Cola which is consumed by more than 1.8 million people around the world. It was invented by John Stith Pemberton in Atlanta, Georgia as a patent medicine. It was introduced on May 8th 1886, thus making it 137 years old. The name coco refers to coca leaves and cola refers to cola nuts, a source of caffeine. The preparation of cola drink is not exactly known. As per Wikipedia, Coca-Cola is sold in many flavours like caffeine, lime, vanilla, and many more (<https://en.wikipedia.org/wiki/Coca-Cola>).

Public toilets are important infrastructure built to provide proper sanitation in cities worldwide. They should be properly maintained to be sterilized and free of pathogenic microorganisms using some strong cleaner, without which it can cause contamination and increase the risk of infection. The chemicals used to clean the toilet are often liquid acid products (Domex, Harpic, Lysol, etc.) used in both diluted and undiluted forms. The composition includes Polycarboxylate, NTA or Trisodium Methyl Glycine Diacetate, Citric acid, Sulfonic, Lactic acid, Formic acid, Isopropanol, and water (<https://www.kviconline.gov.in/pmegp/pmegpweb/docs/commonprojectprofile/ToiletCleaner.pdf>). The main aim is to clean the floor, sink, taps, and the rim and bowl. These are used for cleaning the public toilets as frequently as up to 3 times a week.

The social media information (<https://www.youtube.com/watch?v=U22suBkohC4>) explains the effects of cola as a toilet cleaner, mainly its erosive effects as a cleaner not explaining its antimicrobial properties. This trend was in a boom globally during the initial COVID period (early 2020) as carbonated beverages were more affordable than toilet cleansing agents. Our study mainly aims to check whether coke acts as an antimicrobial agent and can kill or inhibit the growth of microbes that were isolated from public toilets.

According to a few studies^(1,2) conducted on the effects of Coke and other carbonated beverages and their effect on plant growth, it was seen that unsweetened carbonated drinks like soda water might aid in the growth of plants due to the presence of carbon dioxide but are ultimately harmful.

As stated in the study by Adel Alkheldaide *et al.*⁽³⁾ on the chronic effects of soft drink consumption on the health state of Wistar rats and also in the study by D Amato *et al.*⁽⁴⁾ on the acute effects of soft drink consumption to check the calcium and phosphate metabolism in adult

rats. The studies concluded that long periods of coke consumption led to detrimental histological alterations in the bones and livers of rats and also affected the phosphate and calcium metabolism leading to hyperphosphaturia and hypercalciuria.

On testing the antimicrobial effect of concentrated coke on bacteria and germs causing food poisoning like *Escherichia coli*, *Enterococcus faecalis*, *Salmonella enteritidis*, etc. as stated in the study by Şeker Dag *et al.*, it was observed that coke showed considerable antimicrobial effect.

MATERIALS AND METHOD:

In our present study, Coca-Cola was used to check the antimicrobial properties against the culture cultivated from public toilet. It was procured from the supermarket and further work was carried out in the laboratory of Vijaya College, RV road, Bengaluru.

The following is the list of ingredients and nutritional facts present in Coke:

Listed ingredients as per source (<https://en.wikipedia.org/wiki/Coca-Cola>)

- Carbonated water
- Sugar (sucrose or high-fructose corn syrup (HFCS) depending on country of origin)
- Caffeine
- Phosphoric acid
- Caramel colour (E150d)
- Natural flavourings

Nutrition facts as per label for 100ml

- Energy – 44kcal
- Carbohydrate – 10.9g
- Total sugars – 10.6g
- Total fat – 0g
- Total protein – 0g
- Sodium – 8.5mg

Microorganisms isolated from public toilet were cultured in Nutrient agar media which is a general-purpose solid medium which supports the growth of a wide range of microorganisms. It is used to cultivate non-fastidious microorganisms i.e., those organisms that do not require any special nutrient supplementation for their growth. Nutrient agar is one of the most widely used nutrient mediums as it contains many nutrients and is suitable for the growth of several bacteria of *Salmonella* species which are one of the most widely found organisms

in public toilets along with *E. coli* and *Staphylococcus*.

Composition of Nutrient agar media:

1. 0.5% peptone, which acts as a main source of organic nitrogen for bacterial growth.
2. 0.3% beef extract is a water-soluble substance that contains vitamins, carbohydrates, salt, and trace amounts of nitrogen.
3. 0.5% NaCl, helps in maintaining salt concentration in the medium which is similar to the cytoplasmic salt concentration of the microorganisms.
4. 0.5% agar is used as a solidifying agent (If agar is not added, the nutrient broth is obtained).
5. Distilled water, water is essential for all living organisms' growth and provides a medium to transport nutrients.
6. pH is adjusted to 7.4

In our research work, MIC was determined to check the growth of microorganisms.

Minimum Inhibitory Concentration (MIC) was determined to check which concentration of coke had the least growth of microorganisms thereby preparing a dilution series of coke in nutrient broth. The composition of nutrient broth is the same as nutrient agar except that in broth, agar is not added. The nutrient broth was poured into 7 test tubes along with coke in various ratios as shown in Table 1. 1mL of inoculum which was cultured in 0.9% saline was added to all the tubes which were later incubated for 72 hours at 37°C in an incubator (Fig 1).

After 72 hours, the result was checked for the colorimetric absorbance that was measured at 600nm with saline as blank.



Fig1: MIC tubes before incubation

Table 1

Sl no.	The volume of Nutrient Broth (mL)	The volume of Coke (mL)
1.	12	0
2.	10	2
3.	8	4
4.	6	6
5.	4	8
6.	2	10
7.	0	12

After MIC, antimicrobial activity was checked using disk plate method.

In this method, nutrient agar was prepared, poured and allowed to solidify in two sterile petri plates. To this, a loop full of microbes from the parent culture plate (Fig 2) which was cultivated from the public toilet was streaked. Antimicrobial disks were prepared manually using sterilized cotton and soaked in Coke. These disks were later placed onto the agar plates and the plates were incubated at 37°C for 48 hours in an incubator to check for growth and zone of inhibition. The plates were observed later for the results.



Fig2: Parent culture plate cultivated from public toilet

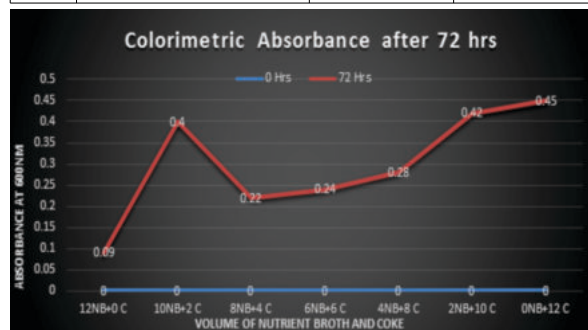
RESULT, OBSERVATION AND DISCUSSION:

In our current study, as tabulated in Table 2, and plotted in Graph 1, the result of MIC was observed after 72 hours of incubation (Fig 3), and it was found that the negative control (tube with 12mL of coke and 0mL of broth) showed the most absorbance of 0.45 at 600nm with least turbidity (turbidity is proportional to the growth of microorganisms), indicating the least growth of microorganism.

Similarly, the positive control (tube with 12mL broth and 0mL coke) showed the least absorbance of 0.09 at 600nm with the most turbidity, thus showing the most growth of the microorganism. The 3 tubes (SL no. 3, 4 and 5 respectively) where the quantity of coke and broth were almost equal i.e., 8mL broth+ 4mL coke, 6mL broth+ 6mL coke, and 4mL broth+ 8mL coke showed very close absorbance values which were quite lower than the negative control, thus indicating a combination of nutrient broth and coke acts as an excellent media for growth of microorganisms.

Table 2: The absorbance at 600 nm

SL no.	The volume of Nutrient Broth (mL)	The volume of Coke (mL)	Absorbance at 600nm
1.	12	0	0.09
2.	10	2	0.40
3.	8	4	0.22
4.	6	6	0.24
5.	4	8	0.28
6.	2	10	0.42
7.	0	12	0.45



Graph 1

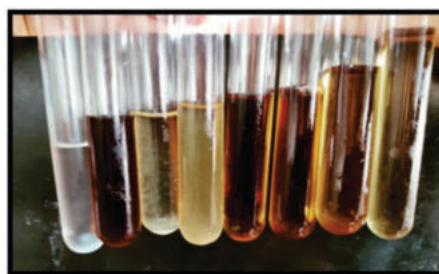


Fig3: MIC tubes after 72 hrs of incubation.

Further, the result was analysed for antimicrobial activity and the plates were checked after 48 hrs (Fig 4 and Fig 5). It was seen that the area around the coke-soaked discs showed excessive and healthy growth of microorganisms in opposition to the zone of inhibition expected.

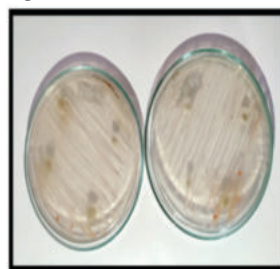


Fig 4: Plates after 48 hrs of incubation.

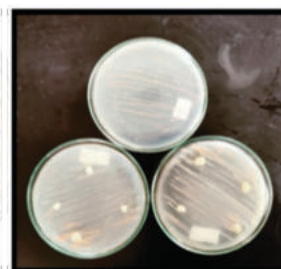


Fig5: Parent culture (top) and cultures with disk (bottom)

The colonies formed in the plates are smooth, round, concave, and pink in colour (Fig 4 and Fig 5). Further the microorganisms were stained (Gram staining) and observed under 100X, they were observed to be Gram-positive cocci.

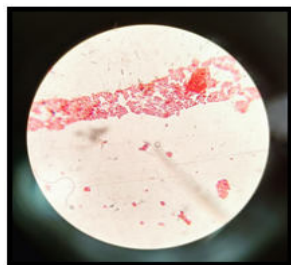


Fig6: Parent culture under 100X

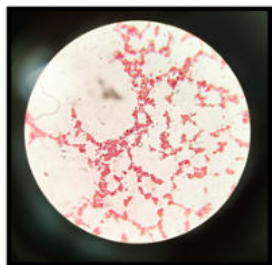


Fig7: Cultures close to disk with coke under 100X

DISCUSSION:

Coca-Cola and other carbonated beverages are one of the most popularly consumed beverages all over the world. Though they are proven to have therapeutic properties when consumed in moderate quantities, excessive consumption is harmful and might even cause necrosis of tissues.

During the COVID lockdown due to the scarcity in availability of groceries and other necessary products due to lockdown, using Coke or Coca-Cola to clean toilets gained a lot of popularity. Different videos were posted on various social media platforms about the different methodologies of using, the time it has to remain in the toilet, and even mixing the coke with other cleansing agents like baking soda and vinegar. These videos showed the cleansing effect of Coke but never once did they discuss the anti-microbial effect.

After this project, we understood the following:

- Cleaning toilets with coke will provide only a temporary solution.
- The toilet might appear clean because of the carbonation of coke and the phosphoric acid present in it but it is not clean concerning the microbial count.
- The major ingredient of Coke is sugar and caramel colour which will act as a great source of nutrition for the microorganisms.

Thus, after cleaning the toilets with coke, it might appear clean but the microorganisms present will not be removed or wiped out like they would be when toilet cleaners are used.

Thus, coke might make a good beverage and might even relieve a person's discomfort when suffering from slight digestive issues but it is not a good cleaning agent, especially not for toilets.

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