

■ ORIGINAL ARTICLE

## Antimicrobial Effect of Different Inks on Microbes

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### ABSTRACT

For ages, the main source of ink for writing documents and books has been natural dyes and vegetable inks. Biochemists have identified these natural inks and dyes that act as antibacterial and anti-fungal agent whereby they protect the historical writings and documents from bacterial or fungal infections. It also acts as moth deterrents. Natural colorants derived from plants have been used for at least 4,000 years as dyes and inks for writing because of their antimicrobial properties. Thus, documents and books written with these inks have survived moisture, humidity, and microbial degradation or deterioration. The type of ink used to write books or documents have forensic relevance. Therefore, the present study was undertaken to understand the antimicrobial properties of older dyes and inks compared to today's inks and their comparative antimicrobial activity. Fountain pen ink and gel pen pigments are water-based but even then their antimicrobial activity have been clearly seen against 5 different bacterial strains in the present study. Against all the bacterial strains, ball pen ink and gel ink demonstrated the greatest Inhibition region. It was also deduced from the results that of all the three, the black ink gave the maximum zone of Inhibition and toxicity to all the five strains of Gram -ve as well as Gram +ve bacterial strain. Thus the study proves that the ink pigments have high antibacterial effect and that both printed and written documents and manuscripts could be preserved without the fear of deterioration.

**KEYWORDS** | ink, documents, colourants, microbes, antimicrobial activity, bacterial strain

### INTRODUCTION

**A** MAJORITY OF TRANSACTIONS IN the world of trade and commerce takes place through written and printed documents. In today's culture, records play a critical role in about half of all cases involving disputed documents. Documents identify any matter conveyed or written or represented on any surface by letters, figures, or signs, or by more than one of these means, intended to be used, or that could be used, as proof of that matter.<sup>1</sup> As a result, determining what kind of ink was used in the writing of that document is critical to determining the age of the record. One of the earliest

forms of forensic science has been the examination of the writing materials. The most difficult task accomplished through this study was determining of the age of the ink. There are a variety of approaches that can be used to determine the age of a newspaper. It could be calculated based on its own state, watermarks, makeup, and other factors.<sup>2</sup>

In certain unusual cases, using a pencil to determine the age of documents can be beneficial. Age of the ink may be used to determine the date of writing. Changes in the color of the ink caused by oxidation and chloride or sulfate diffusion may be

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one of the most effective methods for determining the age of writing was the antibacterial effect of the ink. Ink assessment is also critical for determining if a newspaper belongs to that time period or not. As some inks have characteristics that make them resistant to specific microbes such as antimicrobial markers used throughout the process, more inks have been developed with antimicrobial properties. Germs are the primary cause of the paper's rapid degradation and document damage.<sup>[4]</sup> One of the oldest branches of forensic science was the examination of writing materials, and ink analysis is an important field in forensic science. Inks of ballpoint pens are viscous liquids containing a combination of dyes and pigments, organic solvents, additives such as antioxidants, resins, preservatives, softeners, and trace elements.<sup>5</sup> Ink investigation is a crucial forensic technique that can provide answers to concerns about forged checks, legal contracts, wills, insurance claims, ransom letters and even suicide notes. The primary goal of ink analysis is to determine whether two pieces of written texts were produced using the same ink. To distinguish between different inks used in ballpoint pens, various analytical and spectroscopic techniques are used, and they're typically divided into two distinct categories: destructive/semi-destructive and non-destructive/non-invasive. A portion of the ink sample must be extracted before analysis using destructive/semi-destructive methods. Mass spectrometry combined with various ionization techniques are examples of destructive/semi-destructive procedures that have been used for ink analysis.<sup>6,7</sup> Capillary electrophoresis, Visible spectroscopy VIS, chromatography (thin layer, gas, higher output liquid), Fourier transform infrared spectroscopy, and other techniques for determining elemental composition are some of the techniques used. Non-destructive instruments, on the other hand, refer to ink monitoring on the document using methods that enable the conclusion of ink attributes without affecting the document, which include visual evaluation using a microscope, optical techniques using various lighting sources and filters, luminescence, reflectance IR, and Raman spectroscopy.<sup>8,9</sup>

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## METHODS

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**Sample Collection:** Gel pen ink, Ball pen ink and printer inks were collected from different sources. Old typed and written documents were also collected for the extraction of inks from the documents.

**Isolation of Soil and Water Bacterial Strains:** To study the antimicrobial activity of different dyes and inks, bacterial cultures found in the soil, water and air were isolated. These bacterial cultures are the organisms which would easily grow on old books and documents and would degrade the written or typed inks as well as the cellulose of papers thus, resulting in degradation of important documents.

For isolation of bacterial strains inhabiting air, water and soil, serial dilutions of water and soil samples were made and spread on Nutrient Agar plates. The agar plates were left open in the air for an hour to isolate air microorganisms, and then all of the nutrient agar petri-plates were incubated at 37°C for 24 hours to obtain the relevant bacterial cultures.

**Morphological & Biochemical Characterization of Bacteria:** The isolated bacterial cultures were kept on Nutrient Agar plates and slants for further studies. The morphology of the bacterial cultures was studied by Gram Staining. Gram staining involves Crystal Violet purple staining of Gram +ve bacteria and pink counterstain by Safranin of Gram -ve bacteria. After gram stain the bacterial smears were observed under oil immersion 100X and the shape and color was identified and recorded.

Further characterization and partial identification of the bacterial cultures was done by biochemical tests, performed for each bacteria thus isolated. The biochemical tests performed were: Indole Test, methyl red test, Voges Proskauer test, citrate test, carbohydrates utilizing tests. (Glucose, Maltose, Mannose, Sucrose, Fructose etc.)

**Preparation of Ink Solvent Mixture:** Different types of ink samples were collected from the market to study their antimicrobial effect.

The Inks collected were as follows:

1. Fountain Pen Ink - Black and Blue color

- 2. Ball Pen Ink - Black and Blue color
- 3. Gel Pen Ink - Black and Blue color
- 4. Printer Ink - Black and Blue color

Then these ink samples were dissolved in different solvents like water, methanol, acetone etc., to analyze the extraction of pigments and their specific antimicrobial activity.

**Study of antimicrobial activity of inks and dyes by Agar Well diffusion method:**

Under aseptic conditions, Nutrient Agar was prepared and plated. Using a 6mm diameter Agar medium well cutter, wells of similar length were created and sealed with melted agar. The test organism (isolated bacterial strains) were swabbed on the nutrient Agar plates. After which 80-100µl of ink or dye sample (different solvents) was added to different wells in case of antibacterial assay and related solvents were added to the other wells which are used as control. The swabbed plates with inks were incubated at 37°C for 24 hours. Ink and dye diffusion into the medium inhibits bacterial growth in the vicinity of the source, resulting in the creation of clear “zones” devoid of organism lawn.

**Studying the Antimicrobial effect of ink and printer ink on old documents**

Under aseptic conditions, nutrient Agar was prepared and plated. Small disks were cut from the old documents having pen ink or printer ink on them. The nutrient agar plates were swabbed with the five cultures and then the discs were carefully placed on the swabbed plate upside down to test the antimicrobial activity of the ink present on the old documents. Swabbed plates with inks were incubated for 24 hours at 37°.

**RESULT**

Five bacterial cultures were isolated from the surrounding environment using a serial dilution method and held in pure culture on Nutrient Agar and broth. To characterize and identify the bacterial cultures and understand which bacteria is more resistant, morphological analysis, Gram staining, biochemical tests were done (Table: 1).

Biochemical tests were performed for characterization of the bacterial isolates. The results are shown in Table: 2.

The carbohydrate test was performed by using different types of carbohydrates such as sucrose, fructose, lactose, maltose, mannose, etc.

S. No.	Strain Number	Gram Strain	Morphology Shape	Colony Color
1	Strain 1	Gram Negative	Rod	Pink
2	Strain 2	Gram Positive	Bacillus/rod shape	Purple
3	Strain 3	Gram Positive	Cocci	Purple
4	Strain 4	Gram Positive	Rod shape in chain	Purple
5	Strain 5	Gram Positive	Rod shape	Purple

Table 1 Table showing the shape, morphology and gram-staining of bacterial isolates

Sl. No	Tests	Strain 1	Strain 2	Strain 3	Strain 4	Strain 5
1	Glucose	Negative	Negative/Positive	Negative	Negative	Negative
2	Fructose	Positive	Positive	Positive	Positive	Positive
3	Lactose	Positive	+/-	Negative	Negative	Negative
4	Glucose	Negative	+/-	Negative	Negative	Positive
5	Glucose	Negative	+/-	Negative	Positive	Positive
6	Glucose	Negative	+/-	Negative	Positive	Negative
7	Glucose	Negative	+/-	Negative	Positive	Positive
8	Glucose	Negative	+/-	Negative	Positive	Positive
9	Glucose	Negative	+/-	Negative	Positive	Positive

Table 2 Bio-chemical test result of isolated Isolates

The Gram stain and other biochemical and carbohydrate tests gave a clear identification of the type of genus of the bacterial isolates. On the basis of test results submitted to Bergey’s online software, the isolates were identified as:

- Strain 1** - Enterobacter sps. (85%)
- Strain 2** - Lactobacillus sps. (87%)
- Strain 3** - Staphylococcus aureus (93%)
- Strain 4** - Actinomycetes sps. (89%)
- Strain 5** - Bacillus subtilis (90%)

Once isolated, the bacterial strains were used for analyzing the antimicrobial effect of different inks which were diluted in 3 types of inks—printer ink, ball pen ink, gel pen ink and fountain pen ink—are tested against environmental bacterial cultures. The zone of Inhibition obtained in centimeter when tested against different inks and old documents are shown in Tables 3, and 4 and the comparative zone of Inhibition obtained against black, and blue printer ink, ball pen ink, fountain pen ink, gel pen ink is shown in Figures 1, and 2.

PRINTER INK	DILUTED IN	STRAIN1	STRAIN2	STRAIN3	STRAIN4	STRAIN5
Zone of Inhibition (in centimeter)						
BLACK	Water	3.2	3.1	3.3	3.1	3.1
	Acetone	3.2	3.5	3.3	3.2	3.4
	Methanol	3.3	2.9	2.8	2.8	2.8
BLUE	Water	2.2	2.4	2.4	2.4	2.5
	Acetone	1.3	1.8	2.3	2.1	2.1
	Methanol	2.4	2.3	2.6	2.3	2.6
<b>BALL PEN INK</b>						
BLACK	Water	1.5	0.8	1.1	1.9	1.3
	Acetone	2	2.3	1.9	1.4	1.7
	Methanol	2.3	2.7	2.1	2.7	2.4
BLUE	Water	2.3	2	2	2.1	1.5
	Acetone	3	3.4	3.1	2.9	3
	Methanol	2.9	3.5	3.1	3	3
<b>FOUNTAIN PEN INK</b>						
BLACK	Water	0.9	1.5	1.4	1.2	0.9
	Acetone	0.9	1.4	-	0.9	0.9
	Methanol	1.2	1.2	1.1	1.1	0.8
BLUE	Water	1.2	-	1.2	1.2	1
	Acetone	-	2	1.5	1	0.9
	Methanol	-	-	1.5	0.8	0.9
<b>GEL PEN INK</b>						
BLACK	Water	2.4	2.1	2.4	1.9	-
	Acetone	-	-	-	-	-
	Methanol	2.5	2	2	2.1	1.9
BLUE	Water	2.1	2.1	2.2	2.2	2.3
	Acetone	-	1.9	1.9	1.7	1.9
	Methanol	2.2	2.2	2.4	1.9	2

Table 3 Zone of Inhibition obtained when Printer ink, Ball pen ink, Fountain pen ink, Gel pen ink (Blue & Black) are tested for antimicrobial effect

	STRAIN1	STRAIN2	STRAIN3	STRAIN4	STRAIN5
Zone of Inhibition (in centimeter)					
BIO BOOK 1996	0.5	0.6	0.6	1.1	0.5
BILL BOOK CARBON INK	1	1.1	1.2	0.9	1.1
BILL BOOK PRINTED INK	1.1	0.8	1.2	1	0.8
<b>STRAIN1 STRAIN2 STRAIN3 STRAIN4 STRAIN5</b>					
Zone of Inhibition (in centimeter)					
BOOK (FOUNTAIN INK BLACK)	0.7	1	0.7	-	0.8
BOOK (FOUNTAIN INK BLUE)	1	0.9	0.9	1.3	-
BOOK					
BALL PEN INK	0.9	-	0.9	0.9	0.8

Table 4 Zone of Inhibition obtained when written and typed text of very old Bill books and Text Books are tested for antimicrobial effect on Gel pen ink (Blue & Black) are tested for antimicrobial effect

- The printer ink when dissolved in three different solvents showed varying antimicrobial activity. The black Printer ink gave a better antimicrobial activity having zone of Inhibition from 2.8 cm to 3.5 cm. against different test bacterial isolates. Enterobactersps (gram -ve) gave the highest inhibition zone. The methanol dissolved blue ink gave the best results against all the test microorganisms.
- The ball pen ink when dissolved in three different solvents showed a higher

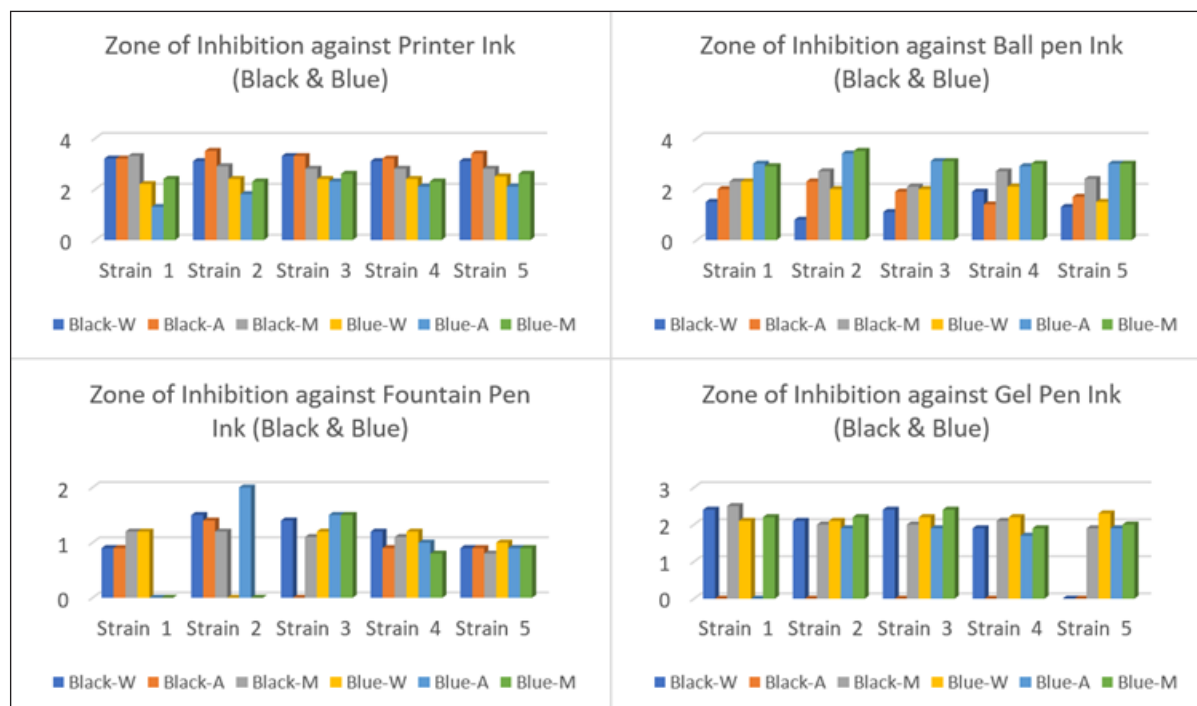


Figure 1 Comparative zone of inhibition obtained against black ink, blue ink, printer Ink, and Ball Pen ink

antimicrobial activity than printer ink. But the Black ball pen ink showed lesser antimicrobial activity as compared to the Blue ball pen ink. The three diluants of Black ball pen ink gave zone of inhibition from 1.5 cm to 2.7 cm against different test bacterial isolates. The least zone of inhibition was shown in case of Lactobacillus strain by black ink dissolved in water. The methanol and acetone dissolved blue ink gave the best results against all the test microorganisms.

- The Fountain Pen ink was observed to be very poor in its antimicrobial activity in both the Blue as well as Black Pigment. The strains Enterobactersps. And Lactobacillus sp. was resistant to both the colored fountain pen inks.
- The Gel Pen ink when dissolved in three different solvents did not show good antimicrobial activity. The three diluants of Blue ball pen ink gave zone of inhibition from 1.7 cm – 2.4 cm against different Test Bacterial isolates. All the bacterial strains were resistant to the Black gel pen ink dissolved in acetone. Thus, the blue ball pen ink pigment has more antimicrobial activity as compared to Black Ink pigment. The methanol and acetone dissolved blue ink gave the best results against all the test

Thus, it is clearly seen and observed through this study that inks and dyes of all kinds are antimicrobial to different extents depending on the dye pigments, which are its constituents. That is why documents and old books and copies are not easily degraded by bacterial cultures even if kept in moist condition for long duration. But, sometimes cellulose digesting fungi can cause degradation to the historical books and documents. The zone of Inhibition observed when discs were used was less as compared to well diffusion, because the concentration of inks on the paper discs was very less

**DISCUSSION**

The color of a pen, printer, or gel ink comes from either a water-soluble dye or a water-insoluble pigment. To make a specific ink, different pigments and dyes like eosin, carbon black, malachite gray, rhodamine, and others are combined with components like Titanium Oxide, Copper Zinc Alloy, or elements like bromine. However, the pigments in gel pen ink are usually copper phthalocyanine and iron oxides, with the gel consisting of water and biopolymers including xanthan gum and tragacanth gum.

Apart from the pigments in the ink, stabilizers,

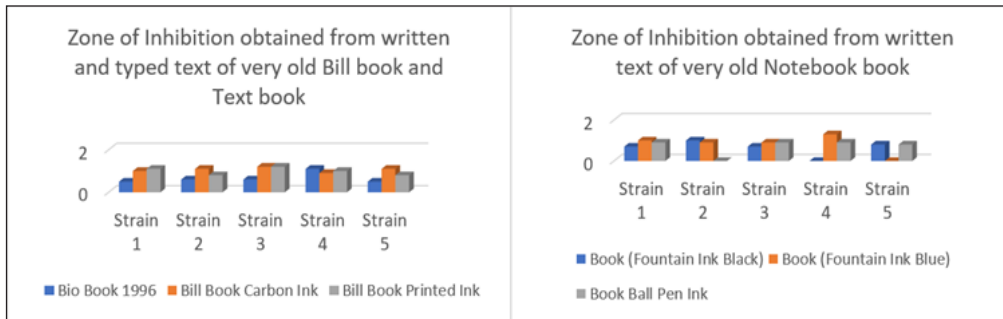


Figure 2 Comparative Zone of Inhibition obtained against black and blue printer ink, Ball Pen

microorganisms.

- When the old books and documents were tested for antimicrobial activity by disc diffusion, it was found that all the inks showed antimicrobial activity against the five test bacterial cultures. The Control discs having only cellulose paper did not give any zone of inhibition. This gave proof that the ink present on the paper discs was actually showing the zone of Inhibition against the Gram +ve and Gram -ve bacterial strains.

polar or non-polar solvents, and other additives such as Glycerides, triethanolamine, and other additives are all present in modern inks.

Ink pigments were derived from vegetables or fungal strains and made in aqueous solutions in ancient times, making the inks more vulnerable to microbe degradation when documents were stored for long periods of time. Inks are particularly toxic to bacteria since they are now made up of mostly organic and synthetic compounds. As a result, when these inks are used, they provide a higher

degree of Zone of Inhibition in almost all cases.

In the present study, different inks such as fountain pen ink, ball pen ink, gel pen ink and printer ink were used to study their effect on the bacterial strains commonly found in places where the books, documents or parchments are stored. The effect of inks and dyes on these bacterial strains are very important since they assess the maintenance and preservation of documents and parchments for longer period, without getting degraded or deteriorated. The antimicrobial activity of the dyes and inks prevents the degradation of documents and also prevent the growth of cellulose-eating or collagen-eating bacterial growth.

When it came to printer ink, the findings showed that black ink had a greater zone of inhibition against all of the bacterial test strains than blue ink. But in all other cases the Blue ink pigments fared better in acting as antimicrobial agent. In case of gel pen black ink, all the bacterial strains proved resistant to the dye as there was no zone of Inhibition. When old and new documents

having ink or printer ink texts were used as antimicrobial discs, it was clearly seen that it was the ink which showed antimicrobial effect instead of the cellulose.

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### CONCLUSION

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Documents play a very important role in our society. Roughly 50% of cases relate to the disputed documents. Documents denote any matter expressed or described upon any surface by means of letters, figures, or marks or by more than one of these means; intended to be used, or which may be used, as evidence of that matter. Hence, it is important to determine what kind of ink was used in writing that document and help in rough estimation of the age of that document. One of the oldest fields of forensic science was the examination of writing materials. Along with this aspect of inks and documents, the aspect of preserving papers, books, copies of documents etc., from bacterial deterioration has to be taken into the account. Thus, antimicrobial inks and dyes are becoming more common and are being manufactured for daily use as well as for use in official documents and papers. **IJFMP**

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