https://www.scirp.org/journal/jbise
J. Biomedical Science and Engineering,
2026, Vol. 19, (No. 3), pp: 141-149

Identification of Bacterial Contaminants of Bananas and Assessment of Their Population Dynamics in Relation to Temperature and Antibiotic Susceptibility

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Keywords: Bacteria, Antibiotics, Banana, Contamination
Received: November 5, 2025 **Accepted:** March 10, 2026 **Published:** March 13, 2026

ABSTRACT

The study aimed to determine the bacterial contamination of banana taken directly from the car that brought banana from the field, and after storage at different temperatures (commercial refrigerator, laboratory refrigerator and lab temperature (35˚C - 38˚C)). The isolated bacteria genera were *Klebseilla pneumonia*, *Klebseilla oxitoca, Bacillus cereus*, *staphylococcus aureus*. The initial bacterial count from a banana sourced directly from the field was 23.2 × 10^6 CFU/ml, compared to 33.65 × 10^6 CFU/ml for a banana obtained from a commercial refrigerator. After five days of incubation at ambient laboratory temperature, the bacterial count increased by 11 × 10^6 CFU/ml. In contrast, samples incubated in a laboratory refrigerator showed a smaller increase, with the count not exceeding 58.11 × 10^6 CFU/ml. The antimicrobial sensitivities show that the tetracycline and ciprofloxacin have more effect to the tree genera, *Klebseilla oxitoca*, *klebsiella pneumonia* and Bacillus cereus (24, 23, 24.33 mm) respectively and Cipro (27, 32, 36.66 mm) respectively, but Novobiocin has an effect but less than tetracycline and ciprofloxacin (11, 13, 10.6 mm). Penicillin has no effect to *Klebseilla oxitoca*, *klebsiella pneumonia,* but has effect to Bacillus cereus (16.7 mm) while the Methicillin has no effect to the tree genera.

1. INTRODUCTION

Bananas are among the most widely consumed fruits globally due to their rich content of essential nutrients, such as vitamins, minerals, and fiber, in addition to their distinct flavor and ease of consumption. However, bacterial contamination of bananas during transportation, storage, or display poses a serious health risk and can serve as a vehicle for transmitting pathogenic microorganisms to humans [1].

Microorganisms surround us from all directions. Some are beneficial and essential for sustaining life on Earth, while others are harmful and cause numerous fatal diseases. Certain microorganisms inhabit soil, water, or air, and some constitute the normal flora in humans or animals. Some microorganisms cause diseases in humans and animals, while others are opportunistic pathogens [2]. Additionally, some contaminate and spoil fruits and vegetables. The sources of contamination can be humans, air, or soil.

Fruits are susceptible to contamination by bacteria and fungi, and the extent of spoilage depends on the degree of infection in the fruit's internal tissues. This can occur during plant growth in the field or during handling. The pH of fruits ranges from 0.5 to 2.3, making them more vulnerable to fungal diseases [3]. Bananas, in particular, are exposed to contamination from various sources.

The first reports of endophytic bacteria in banana trees date back to 1990. Since the year 2000, some progress has been made in isolating and characterizing endophytic bacteria. Several bacterial species have been isolated from bananas [4].

Temperature is one of the most critical factors influencing bacterial growth and reproduction in food. Bacterial numbers increase significantly under warm and humid conditions, while their activity decreases at lower temperatures (Jay *et al*., 2005). On the other hand, testing the sensitivity of bacteria isolated from food to antibiotics is an important aspect of monitoring the development of microbial resistance, which remains one of the greatest challenges to global health [5].

Based on the above, this study aims to identify the types of bacteria contaminating bananas, estimate their numbers under different temperature conditions, and test their sensitivity to certain antibiotics, thereby assessing the health risks associated with handling bananas under various environmental conditions [6].

2. SPECIFIC OBJECTIVES

- Isolate and classify the bacteria present in bananas.
- Calculate the total number of bacterial types present.
- Test the sensitivity of the bacteria to certain antibiotics.

3. MATERIALS AND METHODS

3.1. Equipment

- Oven
- Flasks
- Petri dishes
- Microscope
- Inoculating loop holder
- Swabs
- Graduated cylinder
- Refrigerator
- Tin foil
- Bunsen burner
- Distilled water
- Incubator
- Glass slides and coverslips
- Cotton
- Pasteur pipette

- Test tubes
- Nutrient broth
- Banana samples

3.2. Material

- Culture Media:
  - Nutrient agar
  - Eosin Methylene Blue (EMB) agar
  - MacConkey agar, Blood agar
  - Mannitol salt agar
  - Simmons Citrate agar
  - Mueller Hinton agar
  - Urea agar (or Urea broth)
  - Peptone water
  - Kligler's Iron agar (KIA)
- Reagents:
  - Methyl red
  - Indole reagent (Kovac's reagent)
  - Gram stain

3.3. Sample Collection

Banana samples were collected directly from the vehicle transporting bananas from the field. Approximately 100 banana pieces were gathered and divided into groups, with each group containing 25 pieces. These were distributed across a commercial refrigerator, a laboratory refrigerator, and the laboratory bench. The purpose was to isolate contaminating bacteria from the bananas at different time intervals, identify the bacterial types, and simultaneously estimate the bacterial count in each group.

3.4. Sterilization of Glassware

The glassware was first washed thoroughly and left to dry. It was then sterilized in an oven at a temperature of 181˚C for at least two hours.

Regarding the sterilization of loops and other similar items, they were sterilized by direct exposure to flame after being rinsed in alcohol [7].

3.5. Culture Media

- **Nutrient Broth (Nutrient Agar Oxoid)**
  Used for bacterial cultivation. The medium consists of Lab Lemco Powder, Yeast Extract, Peptone, Sodium Chloride (NaCl), and Agar. The culture medium was prepared according to the manufacturer's instructions by taking ([Amount missing]) and dissolving it in 1 liter of distilled water, adjusting the pH to 6.8. It was sterilized in an Autoclave at 121˚C for 15 minutes [8].
- **Nutrient Agar (N.A)**
  This medium contains the same components as Nutrient Broth but with the addition of 15 - 20 grams of Agar. 28 g of the medium was taken per 1 liter of distilled water and sterilized using the same method as for Nutrient Broth [8].
- **Plate Count Agar**
  Used to determine the total bacterial count via the plate method. The medium contains Yeast Extract, Tryptone, Dextrose, and Agar. 23.3 g was taken per liter of distilled water. After dissolving in a water bath, the pH was adjusted to 7.0, and it was sterilized in an autoclave at 121˚C for 20 minutes. The medium consists of Casein Enzymic Hydrolysate, Yeast Extract, Dextrose, and Agar [8].

- **Eosin Methylene Blue Agar (E.M.B.A)**
  35 g of EMB medium was taken per liter of distilled water, heated using a water bath to dissolve it, and the pH was adjusted to 7.2. It was sterilized in an autoclave at 120˚C for 15 minutes. It consists of Peptic Digest of Animal Tissue, Dipotassium Phosphate, Lactose, Sucrose, Eosin Y, Methylene Blue, and Agar [8].
- **MacConkey Agar (M.C.A)**
  55.04 g of MCA medium was weighed and mixed in 1000 ml of distilled water. It was heated using a water bath, and the pH was adjusted to 7.4 to dissolve it. It was sterilized in an autoclave at 120˚C for 15 minutes. It consists of Peptic Digest of Animal Tissue, Sodium Taurocholate, Neutral Red, Agar, and Lactose [8].
- **Blood Agar Base (B.A.B)**
  21.25 g of the [Note: text says EMB, but context suggests Blood Agar Base] medium was weighed and mixed in 500 ml of distilled water. It was heated using a water bath, and the pH was adjusted to 7.4. It was sterilized in an autoclave at 120˚C for 15 minutes. When the temperature decreased, 7% blood was added.
  It consists of Proteose Peptone, Liver Extract, Yeast Extract, Sodium Chloride, and Agar [8].
- **Mannitol Salt Agar (M.S.A)**
  111.029 g of M.S.A medium was weighed and mixed in 1000 ml of distilled water. It was heated using a Bunsen burner flame to dissolve it and was sterilized in an autoclave at 120˚C for 15 minutes. It is a selective medium for pathogenic *Staphylococcus spp*. [8].
- **Mueller Hinton Agar (M.H.A)**
  38 g of M.H.A medium was weighed and mixed in 1000 ml of distilled water. It was heated using a water bath to dissolve it, and the pH was adjusted to 7.4. It was sterilized in an autoclave at 120˚C for 15 minutes. It consists of Casein Hydrolysate, Beef Extract, Starch, and Agar [8].

3.6. Bacterial Isolation

Samples were taken from bananas transported from the field and from other groups stored at different temperatures for the purpose of bacterial isolation. This was done by swabbing the surface of the banana peel using sterile swabs, first moistened with distilled water, then wiped on the banana surface, followed by streaking onto plates containing different culture media to determine the type.

3.7. Biochemical Tests

- **Citrate Agar Test (C.A)**
  Bacteria are cultured in a medium containing sodium citrate as the sole carbon source. Bacteria capable of utilizing citrate change the color of the medium from green to blue due to the alkaline reaction after incubation for 24 hours. 24.28 g was taken per liter of distilled water, heated using a water bath to dissolve it, and sterilized in an autoclave at 120˚C for 15 minutes.
  This test is used to determine the ability of bacteria to use citrate as an energy source. The medium contains citrate as a carbon source and $NH_4H_2PO_4$ as a nitrogen source. Bacteria that utilize citrate and ammonium salts release ammonia, changing the color of the medium. A positive result is indicated by a blue color [8].
- **Urease Agar Base (U.A)**
  24.01 g of U.A.B medium was weighed and mixed in 1000 ml of distilled water. It was heated using a Bunsen burner flame to dissolve it and was sterilized in an autoclave at 120˚C for 15 minutes. Afterwards, a 40% Urea solution was added aseptically. The medium contains Urea and Phenol Red. If the bacteria produce the urease enzyme, urea is broken down, producing ammonia, which changes the pH to alkaline, turning the color pink (positive). No color change indicates a negative result [8].
- **Peptone Water (P.W)**
  159 g of P.W medium was weighed and mixed in 1000 ml of distilled water. It was heated using a Bunsen burner flame to dissolve it and was sterilized in an autoclave at 120˚C for 15 minutes.

- **Kligler's Iron Agar (K.I.A)**
  57.52 g of K.I.A medium was weighed per liter of distilled water, dissolved using a water bath, and sterilized in the autoclave at 120˚C for 15 minutes. It is a medium used to identify bacteria based on sugar fermentation (contains glucose in the lower part and lactose in the upper part) and H_2S production. A positive test for gas production shows cracking or displacement. A yellow color indicates acid production; red indicates no fermentation. A black precipitate indicates H_2S production [8].
- **Methyl Red Test**
  The Peptone Water medium is inoculated with the bacteria. After 24 hours, a drop of Methyl Red reagent is added. A positive test (+) is indicated by the formation of a red ring [9].
- **Indole Test**
  Indole is a nitrogen-containing compound formed during the breakdown of the amino acid tryptophan. Its production by some bacteria is a differential test. The bacteria are cultured in Tryptone broth. After 24 hours, a drop of Kovac's reagent is added. A positive test (+) is indicated by the formation of a red ring [9].
- **Catalase Test**
  This test is used to identify bacteria producing the catalase enzyme by adding a drop of H_2O_2 to a bacterial colony. A positive test produces gas bubbles (oxygen) [9].
- **Coagulase Test**
  This is a differential test distinguishing *Staphylococcus aureus*, which produces the coagulase enzyme that coagulates plasma, from other species that do not produce this enzyme [9].
- **Gram Stain**
  Bacteria are cultured on Nutrient Agar for 24 hours. They are then examined under a microscope after staining with Gram stain to determine their morphology and reaction to the stain.

3.8. Bacterial Count Estimation

To estimate the bacterial count, small pieces of banana peel (30 grams) were cut and placed in flasks containing 270 ml of sterile distilled water. The flasks containing the banana pieces were shaken using a Vortex mixer. Then, 6 serial dilutions (10^{-1} to 10^{-6}) were prepared. From each dilution, 1 ml was taken, poured into a sterile Petri dish, and about 20 ml of Plate Count Agar was added to estimate the bacterial count in each group.

3.9. Antibiotic Sensitivity Testing of Bacteria

For the antibiotic sensitivity test on the isolated bacterial types, the bacteria were cultured on Mueller Hinton Agar (Muller Hinton agar). This was done by taking the bacterial sample and preparing a suspension in physiological saline. A portion of the suspension was taken using a sterile swab and spread evenly on a plate containing Mueller Hinton medium. Antibiotic discs containing Novobiocin (5 mm), Penicillin (10 mm), Tetracycline (30 mm), Methicillin (5 mm), and Ciprofloxacin (5 mm) were then placed on the agar. After 24 hours, the inhibition zones were measured in millimeters (mm). Results are shown in **Table 1**.

Table 1. The sensitivity of the bacteria to antibiotics.

Bacteria	T (30 mg)	C (5 mg)	N (5 mg)	P (10 mg)	M (5 mg)
K. oxytoca	24	36.33	10.6	0	**0**
k. pneumonea	23	32	13	0	**0**
B. cereus	24.33	27	11	16.66	0

(T) Tetracycline; (N) Novobiocn; (P) Penicillin; (M) Methicillin; (C) Ciprofloxacin.

4. RESULTS AND DISCUSSION

This study aimed to isolate and identify the types of bacteria contaminating bananas, and to investigate the effect of temperature on them as well as their antibiotic sensitivity. Samples were taken directly from the vehicle transporting bananas from the field to the central market and were divided into groups: one group was placed in a commercial refrigerator (temperature 18˚C - 25˚C), another group in a laboratory refrigerator (4˚C), and a third group was kept in the laboratory (temperature 35˚C - 38˚C). Various microscopic and biochemical tests were conducted on these groups, including the group transported directly from the field.

The following species were isolated: *Klebsiella pneumonia*, *Klebsiella oxytoca*, *Bacillus cereus*, and *Staphylococcus aureus*. The results indicated that *K. oxytoca* constituted the highest proportion (55%) of the bacteria isolated from the bananas, followed by *B. cereus* (27%), with *K. pneumonae* and *Staphylococcus aureus* present in equal proportions (9% each) (Table 2 and Figure 1).

The genera Klebsiella and Bacillus were identified. Rahman (2016) isolated K. pneumoniae, Staphylococcus aureus, and *K. oxytoca*, while Mai (2016) and Martinez (2003) isolated *Klebsiella* spp. Notably, Azis (2012), Adolf (2012), and Sebastien (2013) isolated Staphylococcus aureus.

Regarding the bacterial cell count (CFU/ml) contaminating the bananas—measured immediately after transport from the field, after storage at different temperatures for five days (in a commercial refrigerator at 18˚C - 25˚C), a laboratory refrigerator at 4˚C, and at laboratory room temperature of 35˚C - 38˚C). The results show the bacterial load (CFU/ml) in bananas stored under varying temperature conditions compared to the initial count upon direct collection from the field. The effect of storage temperature on bacterial count is illustrated in Table 3 and Figure 1.

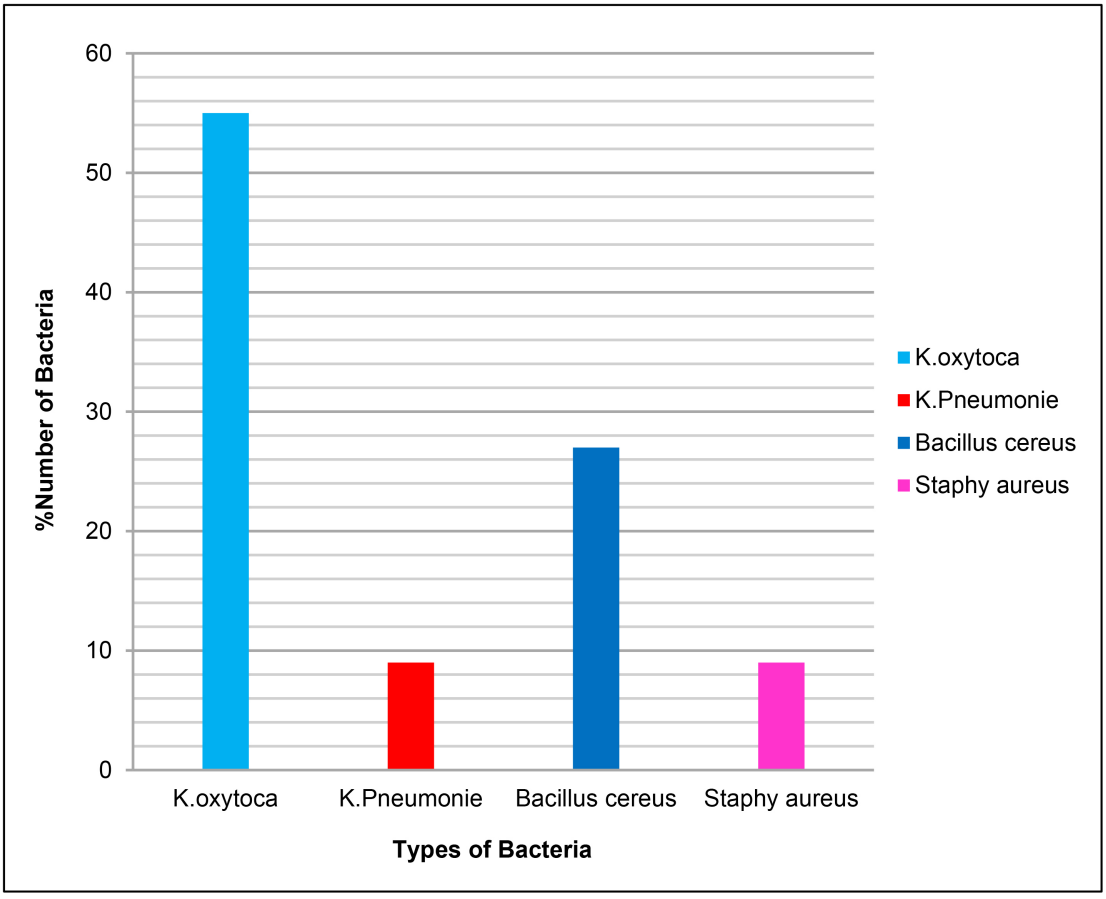

Figure 1. The types of bacteria isolated from bananas and the percentage of each.

Table 2. **(a) The biochemical tests performed on the bacteria isolated from the bananas; (b) the tests performed on *Staphylococcus aureus.***

(a)

NO	Bacteria (strains)	SH	KIA		C	U	I	MR	GAS	G
			BUT	SLOP						
1	*K. oxytoca*	R	Y	Y	+	+	+	−	+	−
2	*K. pneumonae*	R	Y	Y	+	+	−	−	+	−
3	*Bacillus cereus*	R	Y	Rd	+	+	−	−	−	+

Key word: Y. yellow; C. citrate; U. urease; G. gram stain; Sh. Shape; Rd. red; I. indole; (−) negative; (+) positive; MR. methyl red.

(b)

NO	*Bacteria*	G	SH	Tests
4	*Staphylococcus aureus*	+	Grape shape cocci	**Catalase (+)** **Coagulase (+)**

Key word: sh. Shape; G. gram stain; (+) positive.

Table 3. **Effect of temperature on the count of bacteria contaminating bananas at different storage temperatures.**

No.	Sample Location	Bacterial Count
1	Bacteria count in bananas transported directly from the field	2.32×10^6 CFU/ml
2	Bacteria count in bananas stored in the commercial refrigerator (18˚C - 25˚C)	3.365×10^4 CFU/ml
3	Bacteria count in bananas after storage in the laboratory (35˚C - 38˚C)	11×10^6 CFU/ml
4	Bacteria count in bananas after storage in the laboratory refrigerator (4˚C)	5.811×10^6 CFU/ml

As for the bacterial count in bananas transported directly from the field, it was approximately 2.3×10^6 CFU/ml. This is attributed to contamination from environmental factors such as dust and soil, the lack of health awareness among many workers involved in harvesting and collecting bananas, and the failure to use gloves during work.

Regarding the banana samples placed in the laboratory (at a temperature of 35˚C - 38˚C), the bacterial count was approximately 11×10^6 CFU/ml, indicating a significant increase. The laboratory temperature is suitable for bacterial growth and falls within the optimal range, which explains this substantial rise.

The bacterial count in the banana samples stored in the laboratory refrigerator (at 4˚C) was approximately 5.8×10^5 CFU/ml. It is noteworthy that there was an increase compared to the count in bananas transported directly from the field, but a decrease compared to the count in bananas stored in the laboratory. This is because the refrigerator temperature inhibits bacterial growth.

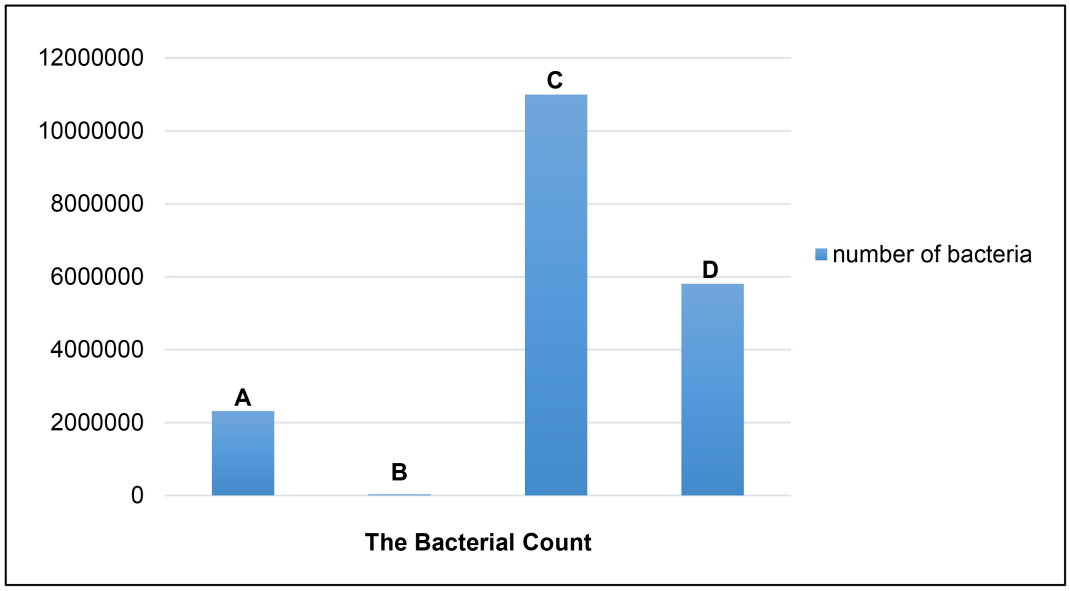

Figure 2. The bacterial counts preserved at different temperatures compared to the count immediately after being transported from the field.

As for the bananas stored in the commercial refrigerator, the bacterial count decreased significantly to approximately 3.4×10^4 CFU/ml (Figure 2). It was observed that the bananas in the commercial refrigerator were treated with a spray of 90% ethanol. It is known that spraying bananas with ethanol alcohol gives them a yellow color and promotes ripening. Since ethanol is an alcohol that inhibits and kills bacteria, the bacterial count decreased drastically. Additionally, the temperature of the commercial refrigerator (18˚C - 25˚C) is lower than the laboratory temperature, which is considered optimal for bacterial growth.

Regarding the antibiotic Novobiocin, it also exhibits equal effectiveness against the three species—*K. pneumoniae*, *K. oxytoca*, and *B. cereus*—with inhibition zone diameters of 11 mm, 13 mm, and 11 mm, respectively (Table 1, Figure 3). However, its effect is less pronounced compared to Tetracycline and Ciprofloxacin.

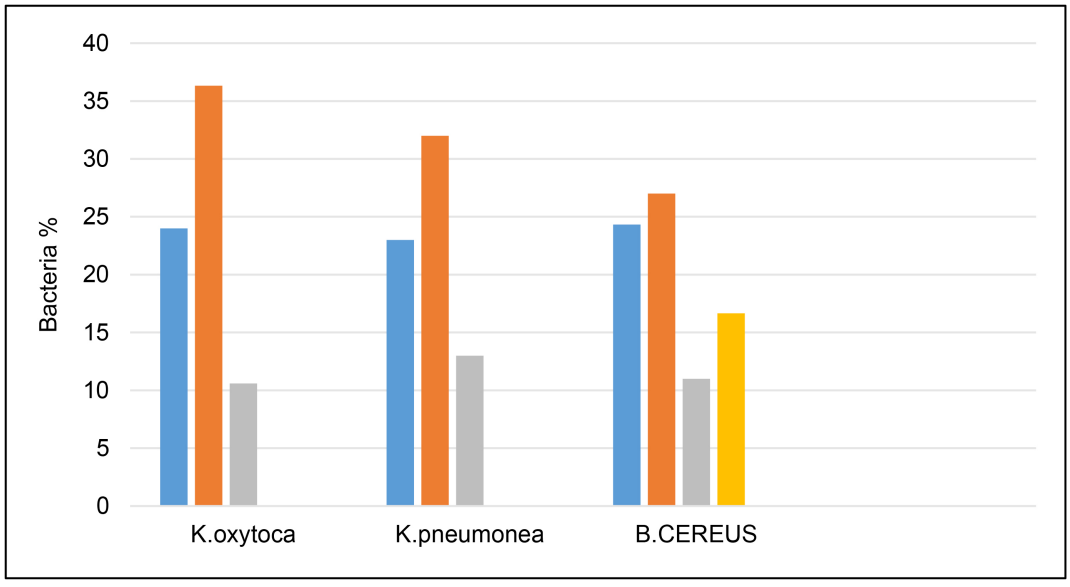

Figure 3. The percentage (%) of antibiotic sensitivity of the bacterial species.

As for Penicillin, it shows no effect on the two *Klebsiella* species (*K. pneumoniae* and *K. oxytoca*) but has a noticeable effect on *Bacillus cereus*, with an inhibition zone diameter of 16.7 mm. In the case of Methicillin, it demonstrates no effect on any of the three species.

5. RECOMMENDATIONS

- Bananas should be transported using hygienic methods and should, as much as possible, be prevented from coming into contact with soil.
- Gloves must be worn when unloading bananas from vehicles and when removing them from refrigerators.
- Hygiene rules for workers must be followed.
- Hygienic facilities should be established for selling vegetables and fruits.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest regarding the publication of this paper.

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