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Original Research Article

Bacteriological analysis of plastic and wood chopping boards

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Abstract: This study was conducted to compare the microbial load of used plastic and wood chopping board samples and to determine the presence of Salmonella sp. and other Enterobacteriaceae species from the samples. Plate counts on Nutrient Agar and Mc Conkey agar were conducted to determine total microbial load and number of presumptive Salmonella colonies, respectively. Cultural characterization of isolates was done based from the 8th Edition of Bergey's Manual for Determinative Bacteriology. Identification of Salmonella sp. and other Enterobacteriaceae was done using standard microbiological techniques involving the use of selective and differential media. Gram staining was performed to confirm morphological characteristics of isolates. Results of the study revealed that used plastic chopping boards have a significantly higher total microbial load than wood chopping boards with a mean of $1.41x10^7$ and $1.28x10^7$, respectively. The number of presumptive Salmonella sp. was also significantly higher in plastic chopping boards than wood chopping boards with a mean of $4.25x10^6$ and $1.6x10^4$, respectively. Colonies isolated from both chopping boards showed various cultural characteristics, such as size, pigmentation, form, margin and elevation. Successive confirmatory biochemical tests on presumptive Salmonella sp. isolates confirmed the presence of Salmonella sp. and other Enterobacteriaceae sp. such as Enterobacter, Klebsiella, Escherichia, Citrobacter, Arizona and Proteus. The presence of rod-shaped and pinkish cells from Gram-stained isolates reveals the Gram-negative bacteria and further confirms the presence of Salmonella sp. and other Enterobacteriaceae sp.
Keywords: Salmonella sp., Plastic chopping boards, Wood Chopping Boards, agar, incubation, Gram stain.

INTRODUCTION

The incidence of food borne illness in the United States each year has been estimated to be between 24 and 81 million cases [1]. Food borne illness results in up to 9,000 deaths annually [2] .Food borne illness originates in food service establishments, in the home, in the food processing industry and from unknown breakdowns. Of those with identified sources, 79% originated in commercial facilities and 21% originate at home. While consumers are very concerned about food safety in general terms, they are much less concerned about microbiological hazards [3]. In addition, they generally have inadequate knowledge about measures to prevent food borne illness at home, only 54% of consumers said they would wash a cutting board with soap and water after chopping fresh meat and before cutting fresh vegetables or fruits for their salads [4].

Cutting boards used to prepare raw meat can be used to prepare salad or other uncooked food, transferring disease-causing bacteria and other agents from the meat to the salad. Wooden cutting boards have been widely used for centuries, while boards made from various polymers have been available since the early 1970s. Glass cutting boards are third option to consumers.

Research has shown that when bacteria were inoculated on both wooden and polymer boards, bacterial recoveries from wooden boards generally were less than those from plastic boards, regardless of a new or used status [5]. On the other hand, some countries exhort cooks to avoid porous, organic and germ encouraging wood in favor of inert sterilizable plastic chopping boards. Other countries still prefer to use wooden chopping boards because they claim that wood has mysterious natural antibiotic effect during their tree life and even now as they are being chopped upon.

Objectives of the Study

Generally, this study aims to determine and analyze the bacteria present in plastic and wooden chopping boards.

Specifically, this study aims to:

1. Determine cultural characteristics of colonies on Nutrient agar.
2. Compare the total bacterial load of plastic and wooden chopping board for three days.
3. Compare the number of presumptive Salmonella sp. per cm^2 in plastic and wooden chopping board.
4. Isolate and identify Salmonella sp. from plastic and wood chopping board using standard microbiological techniques.

5. Determine the morphological characteristics and Gram stain reaction of Salmonella sp. isolates.

Significance of the Study

The bacteriological analysis of plastic and wooden chopping boards was conceptualized in order to come up with data which will serve as source of information to the public as to which chopping board is more advisable to use, be it in home or in business.

Scope and Limitation

This study was limited only on the determination and analysis of microorganisms of Family Enterobacteriaceae present in plastic and wooden chopping board specifically the Salmonella sp. Identification of other bacteria were not included in this study and is limited only on the cultural characterization of colonies. Wooden chopping boards from tamarind (Tamarindus indica) tree and thermoset type of plastic chopping boards were the only chopping boards considered in the study. Swab samples were collected for three consecutive days.

MATERIALS AND METHODS

Materials

The materials used in the conduct of the study were five thermoset plastic and five wooden chopping board, incubator, colony counter, stirring rod, glass L-rod, petri dish, pipette, test tubes, test tube rack, aluminium foil, alcohol lamp, match, inoculating loop, inoculating needle, cotton plugs, vortex shaker, graduated cylinder, beaker, flask, bactopeptone solution, Nutrient Agar, Mc Conkey Agar, Salmonella-Shigella agar, Triple Sugar Iron Agar, Citrate Agar and Lysine Iron Agar, Gentian Violet, Gams's Iodine, ethyl alcohol and saffranine.

Collection and Preparation of Samples

Five pieces of plastic and wood chopping boards were collected from different houses in Tuguegarao City; it was wrapped in aluminium foil to avoid contamination from dirt and dust during transportation. The swab technique was used to collect samples which covered an entire surface of $1cm^2$. Swab samples were collected for three days.

Sterile bactopeptone water was prepared as dilution blank and serial dilution of the samples we 10^{-2}, 10^{-4} and 10^{-6}. Each swab sample was transferred to 99ml of dilution blanks and labelled as 10^{-2} dilution. A 1ml aliquot from 10^{-2} dilution was transferred to 99ml dilution blank and labelled as 10^{-4} dilution. From 10^{-4} dilution, 1.0ml aliquot was transferred to 99ml and labelled as 10^{-6} dilution.

Microbial Population Determination

The spread plate technique was used to determine microbial population. One ml of each dilution was plated in sterile Nutrient Agar in three replicates. The inoculums were spread using glass L-rod. The plates were incubated at 37°C for 24 hours in an inverted position. This position prevents condensation of moisture in the surface of the agar medium during incubation period.

Colony Count

Colony counting was done in plates with 30 to 300 colonies. The number of cells per ml (cfu/ml) was computed by multiplying the average of duplicate plate counts by the dilution factor divided by volume aliquot plated.

$$\text{Colony forming unit/ml} = \frac{\text{Average x d.f.}}{\text{Volume plated}}$$

Where:
Average = average number of colonies
d.f. = dilution factor
Volume plated = volume of aliquot plated

Characterization of Colonies

Cultural characteristics of colonies on Nutrient Agar was described using the criteria set in the Bergey's Manual for Determinative Bacteriology, 8th Edition., 1995[6].

Isolation and Identification of Salmonella sp.

A) Primary Culture

Using aseptic technique, 1ml of each serial dilution was spread on sterile Mc Conkey Plates in three replicates. Inoculum was spread using glass L-rod. Plates were incubated at 40°C for 18 to 24 hours to permit growth of the colonies. This step favoured the recovery of Salmonella sp.

b) Selective Media

After incubation, a loopful of cells from the Mc Conkey plates were streaked on Salmonella-Shigella Agar and incubated at 35°C for 24±2 hours to permit the slow growth of Salmonella sp. Numbers of presumptive Salmonella colonies were noted.

C) Confirmatory Biochemical Test

Typical or suspicious colonies from the Salmonella-Shigella Agar were collected and inoculated on Triple Sugar Iron slant (TSI Agar). In inoculating TSI Agar, aseptic technique was considered, the butt were stabbed using inoculating needle and the slanted area were streaked using inoculating loop. Slants were incubated at 35°C for 24±2 hours. Reactions on the butt and slanted area were noted.

After incubation, loopful of culture from TSI agar were stabbed and streaked on Lysine Iron Agar (LIA) slants. After 24 hours, color change was noted.

The next day, loopful of cultures from LIA was stabbed and streaked on Citrate Agar slant. Color change was noted after incubation.

Gram Stain

The most useful staining in bacteriology is Gram's Differential Stain. Practically all bacteria can be classed as Gram positive or Gram negative, depending on whether the original stain is fixed on the organism.

Salmonella colonies from the Lysine Iron Agar slants were collected and fixed in a microscope slide. The slide was flooded with Gentian Violet for three minutes and rinsed with distilled water. Afterwards, it was covered with Gram's Iodine solution for two minutes. The slide was decolorized with 95% ethyl alcohol until the color ceased to run from preparation, which took up 30 seconds. Then, it was counter stained with saffranine for 30 seconds.

RESULTS AND DISCUSSION

Cultural Characteristics of Colonies in Nutrient Agar

Isolates from plastic chopping boards showed colonies ranging from small, moderate, and large. Different kinds of pigmentation such as yellow, orange and white were also observed. Variations in forms were also prominent. Some were circular with unbroken peripheral edge, some were irregular with edges that have peripheral indention and some were rhizoid in form with root like spreading growth. According to margin, colonies also exhibited heterogenous type. Some colonies were entirely even while others have undulate type of outer edge with wavy indentions, and in some, colonies appeared to have filamentous or threadlike outer edge. As to elevations or degree to which the colony is raised, colonies showed variety of types. Some were flat while others were slightly elevated. Dome shaped elevation and raised growth with elevated convex central region were also observed.

On the other hand, isolates from wood chopping boards showed varying sizes ranging from small, moderate, to large, while all colonies were white. In form, colonies showed unbroken peripheral edges with an entirely defined outer edge. Elevations varied from flat to slightly elevated colony growth.

Bacterial Load of Plastic and Wood Chopping Boards

Generally, bacterial load in the plastic chopping board samples showed a decreasing trend of growth during the three day sampling except for P1 and P2. P4 had the highest total microbial load on Day1 with $1.73x10^{7}$ cfu/ml whereas P3, P2, P5 and P1 have $1.67x10^{7}$, $1.59x10^{7}$, $1.55x10^{7}$ and $1.47x10^{7}$ cfu/ml, respectively (Table 1).

Table 1:Two-Way ANOVA of cfu/ml of used Plastic and Wood Chopping Boards on Nutrient Agar

Source of Variance	Sum of Squares	d.f.	Mean Square	F Ratio	Prob
Treatment	$1.21x10^{13}$	1	$1.21x10^{13}$	6.42	0.18*
Day	$5.01x10^{13}$	2	$2.50x10^{13}$	13.2	$1.34x10^{4}$**
Interaction	$4.49x10^{12}$	2	$2.25x10^{12}$	1.18	0.32
Error	$4.54x10^{13}$	24	$1.89x10^{12}$		
Total	$1.12x10^{14}$	29			

** Highly significant
*significant

Table-2:Two-Way ANOVA of cfu/ml of Presumptive Salmonella sp.in used Plastic and Wood Chopping Boards on Mac Conkey Agar

Source of Variance	Sum of Squares	d.f.	Mean Square	F Ratio	Prob
Treatment	$1.34x10^{14}$	1	$1.34x10^{14}$	1577.75	0.00**
Day	$2.33x10^{11}$	2	$1.16x10^{11}$	1.37	0.27
Interaction	$2.02x10^{11}$	2	$1.01x10^{11}$	1.19	0.32
Error	$2.04x10^{12}$	24	$8.50x10^{10}$		
Total	$1.37x10^{14}$	29			

** Highly significant

Table-3: Total Microbial Load (cfu/ml) of Used Plastic Chopping Board Samples on Nutrient Agar

Day	(cfu/ml)				
	P1	P2	P3	P4	P5
1	$1.47x10^{7}$	$1.59x10^{7}$	$1.67x10^{7}$	$1.73x10^{7}$	$1.55x10^{7}$
2	$1.50x10^{7}$	$1.65x10^{7}$	$1.29x10^{7}$	$1.49x10^{7}$	$1.24x10^{7}$
3	$1.20x10^{7}$	$1.30x10^{7}$	$1.26x10^{7}$	$1.04x10^{7}$	$1.17x10^{7}$

Mean= 1.41 $x10^{7}$

The lowest microbial load on Day 3 with $1.04x10^7$ cfu/ml was observed on P4. On the other hand, replicates P5, P1 and P2 had $1.17x10^7$, $1.20x10^7$, $1.26x10^7$ and $1.30x10^7$ cfu/ml, respectively.

A decreasing trend of microbial load was similarly observed from wood chopping board samples (labelled W1, W2, W3, W4 and w5) (Table 2). W2 had the highest microbial load with $1.85x10^7$ cfu/ml whereas W4, W3, W5 and W1 have $1.36x10^7$, $1.35x10^7$, $1.33x10^7$ and $1.23x10^7$ cfu/ml, respectively.

Table- 4: Total Microbial Load (cfu/ml) of Used Wood Chopping Board Samples on Nutrient Agar

Day	(cfu/ml)				
	W1	**W2**	**W3**	**W4**	**W5**
1	$1.23x10^7$	$1.85x10^7$	$1.35x10^7$	$1.36x10^7$	$1.33x10^7$
2	$1.23x10^{7:}$	$1.17x10^7$	$1.23x10^7$	$1.33x10^7$	$1.28x10^7$
3	$1.16x10^7$	$1.18x10^7$	$1.17x10^7$	$1.18x10^7$	$1.25x10^7$

Mean = $1.28x10^7$

W1 contained the lowest microbial load on Day 3 of $1.16x10^7$ cfu/ml. W3 contained a microbial load of $1.17x10^7$ cfu/ml, while W2 and W4 had $1.18x10^7$ cfu/ml each. W5 had $1.25x10^7$ cfu/ml.

Mean computation showed that microbial load in plastic chopping boards is higher than wood with 1.41 $x10^7$ and $1.28x10^7$ cfu/ml, respectively. Results of Analysis of Variance revealed a significant difference on the total microbial load of two chopping boards at 5% level of significance (Appendix A. Table 2) suggesting that wood chopping board can support least microbial growth. It is probable that they may contain some inhibitory substances that are destructive to microorganisms [7].

However, no interaction was observed on the total microbial load from the different chopping boards and the number of days of collection (Appendix A, Table 3) suggesting that the time of sampling has no effect on the total microbial load of the chopping boards.

Primary Culture using Mc Conkey Agar

Colonies of Enterobacteriaceae appeared on Mc Conkey plates after 24 hours of incubation at 40°C. Colonies regarded as presumptive Salmonella sp. were circular in shape and exhibited whitish-colourless colonies. The numbers of presumptive Salmonella sp. (cfu/ml) in plastic and wood chopping board samples are presented in Tables 3 and 4, respectively.

Table 5 reveals that P3 had the highest number of presumptive Salmonella sp. on Day1 of sample collection with $5.23x10^6$ cfu/ml followed by P1, P2, P4 and P5 with $4.63x10^6$, $4.33x10^6$ and $3.93x10^6$ cfu/ml, respectively.

Table 5: Colony forming unit (cfu/ml) of presumptive Salmonella sp. in Plastic Chopping Board Samples on Mc Conkey Agar

Day	(cfu/ml)				
	P1	**P2**	**P3**	**P4**	**P5**
1	$4.63x10^6$	$4.33x10^6$	$5.23x10^6$	$4.03x10^6$	$3.93x10^6$
2	$4.30x10^6$	$4.50x10^6$	$4.63x10^6$	$4.30x10^6$	$3.70x10^6$
3	$3.73x10^6$	$3.77x10^6$	$4.10x10^6$	$3.97x10^6$	$4.53x10^6$

Mean = $4.25x10^6$

P1 and P3 showed decreasing pattern of growth of presumptive Salmonella sp. during the sampling period, whereas an increase in the number of presumptive Salmonella sp. Was observed on Day 2 for P2 and P4. An increase from $4.33x10^6$ to $4.50x10^6$ cfu/ml was observed for P2 while an increase from $4.03x10^6$ to $4.30x10^6$ cfu/ml was observed from P4.

On Day3, presumptive Salmonella sp. in P5 was highest with $4.53x10^6$ cfu/ml. On the other hand, P1contained the lowest number of presumptive Salmonella sp. with $3.73x10^6$ cfu/ml. P2, P4 and P3 have $3.77x10^6$, $3.97x10^6$ and $4.10x10^6$ cfu/ml, respectively.

Table 6 presents the number of presumptive Salmonella sp. isolated from wood chopping board samples. Only W1 and W3 revealed the presence of presumptive Salmonella sp. isolates. No evidence for the presence of presumptive Salmonella sp. was observed on W2, W4 and W5 since only pinkish colonies grew on Mc Conkey plates. W1 contained higher number of presumptive Salmonella sp. than W3, with $6.67x10^4$ and $1.0x10^4$ cfu/ml, respectively on Day1 of sample collection.

Table 6: Colony forming unit (cfu/ml) of presumptive Salmonella sp. in wood chopping board samples on Mc Conkey Agar

Day	cfu/ml				
	W1	W2	W3	W4	W5
1	$6.67x10^4$	0	$1.0 x10^4$	0	0
2	$1.33 x10^4$	0	$1.0 x10^4$	0	0
3	$1.0 x10^4$	0	$1.0 x10^4$	0	0

Mean= $1.6 x10^4$

A decreasing pattern of presumptive Salmonella sp. was observed on W1. W3 showed consistency in growth with $1.0 x10^4$ cfu/ml from Day 1 to Day3 collection.

In general, plastic chopping board samples contained higher colony forming unit per ml of presumptive Salmonella sp. than that of wood chopping board samples. Mean computations showed $4.25x10^6$ and $1.64 x10^4$ cfu/ml of presumptive Salmonella sp. on plastic and wood chopping boards, respectively. Analysis of Variance revealed highly significant difference on cfu/ml of presumptive Salmonella sp. of the two chopping boards at 1% level of significance, suggesting that more Salmonella sp. can grow on plastic chopping boards rather than wood chopping boards.

However, no interaction was observed on the number of presumptive Salmonella sp. from the different wood chopping boards and the number of days of collection suggesting that time element for sample collection did not affect the number of Salmonella sp. colonies that grew in the agar.

Selective Media using Salmonella-Shigella Agar

Suspected Salmonella sp. colonies sub cultured from Mac Conkey plates reacted positively on SSA. Pinkish to translucent colonies characterized with black centres appeared after 24 hours of incubation at 35°C. Blackening of some portion of the medium was a reaction of Salmonella sp. to one of the components of the medium, sodium thiosulfate, a substance for H_2S production.

Confirmatory Biochemical Test

A summary of the results of confirmatory biochemical reactions of presumptive Salmonella sp. isolates from plastic and wood chopping board samples to Triple Sugar Iron Agar and Citrate Agar are presented in Tables 5 and 6, respectively.

Triple Sugar Iron is a medium that measures the ability of the bacterium to utilize three sugars, namely, glucose, sucrose and lactose, the concentration of which are 0.1%, 1.0% and 1.0%, respectively. A ph indicator included in the medium detects acid production from fermentation of these carbohydrates. A yellow color change indicates acid in the medium while no color change indicates alkalinity. Carbohydrate utilization could also be determined through the analysis of the extent of acid production. Acid production limited only to the butt of the tube is indicative of glucose utilization. This is because the concentration of glucose is lower than that of other sugars, thus the acid production is not very extensive. Acid production in the slant and butt indicates sucrose or lactose fermentation because of the relatively high concentrations of these sugars, thus leading to extensive acid production. Since these substances are present in high concentration, they serve as substrates for continued fermentative activities with maintenance of an acid reaction in both slant and butt. TSI agar can also detect reduction of thiosulfate to hydrogen sulphide and production of other gases. Hydrogen sulphide production will turn parts of the agar black. Production of other gases is marked by cracks in the agar as well as an air gap at the bottom of the test tube.

The results of biochemical tests of isolates from plastic chopping board samples are presented in Table 7. Isolates from P1, P2 and P5 produce alkaline over acid reaction with gas and hydrogen sulphide production while isolates from P3 and P4 showed acid over acid reaction with or without hydrogen sulphide production.

Similarly, the presumptive Salmonella sp. isolates from wood chopping board samples showed contrasting biochemical reactions on the three differential media (Table 8). Generally, isolates from W1 showed acid over acid reaction, with and without production of Hydrogen Sulphide while isolates from W2 showed alkaline over acid reaction with gas and Hydron Sulphide production.

Table 7: Selective and Biochemical Reactions of Suspected Salmonella sp. Isolates from Plastic Chopping Board

Chopping Board Sample	Replicate	Biochemical Reactions					Organism
		SSA	**TSI**	**H2S**	**LIA**	**Citrate**	
P1	1	+	alk/a	+	+	+	Salmonella sp.
	2	+	alk/a	+	+	+	Salmonella sp.
	3	+	alk/a	+	+	+	Salmonella sp.
P2	1	+	alk/a	+	+	+	Salmonella sp.
	2	+	alk/a	+	+	+	Salmonella sp.
	3	+	alk/a	+	+	+	Salmonella sp.
P3	1	+	a/a	-	+	+	Escherichia, Klebsiella and Enterobacter
	2	+	a/a	+	+	+	Citrobacter, Arizona and Proteus
	3	+	alk/a	+	+	+	Salmonella sp.
P4	1	+	alk/a	-	+	+	Salmonella sp.
	2	+	a/a	+	+	+	Citrobacter, Arizona and Proteus
	3	+	a/a	+	+	+	Salmonella sp.
P5	1	+	alk/a	+	+	+	Salmonella sp.
	2	+	alk/a	+	+	+	Salmonella sp.
	3	+	alk/a	+	+	+	Salmonella sp.

Table 8: Selective and Biochemical Reactions of Suspected Salmonella sp. Isolates from Wood Chopping Board

Chopping Board Sample	Replicate	Biochemical Reactions					Organism
		SSA	**TSI**	**H2S**	**LIA**	**Citrate**	
W1	1	+	alk/a	+	+	+	Salmonella sp.
	2	+	a/a	+	+	+	Escherichia, Klebsiella and Enterobacter
	3	+	a/a	-	+	+	Citrobacter, Arizona and Proteus
W2	1	+	a/a	-	+	+	Citrobacter, Arizona and Proteus
	2	+	alk/a	+	+	+	Salmonella sp.
	3	+	alk/a	+	+	+	Salmonella sp.
W3	1	+	a/a	-	+	+	Escherichia, Klebsiella and Enterobacter
	2	+	a/a	+	+	+	Citrobacter, Arizona and Proteus
	3	+	alk/a	+	+	+	Salmonella sp.
W4	1	+	alk/a	-	+	+	Salmonella sp.
	2	+	a/a	+	+	+	Citrobacter, Arizona and Proteus
	3	+	a/a	+	+	+	Salmonella sp.
W5	1	+	alk/a	+	+	+	Salmonella sp.
	2	+	alk/a	+	+	+	Salmonella sp.
	3	+	alk/a	+	+	+	Salmonella sp.

Alkaline over acid reaction with gas and H_2S production is a characteristic of Salmonella sp. Acid over acid reaction with H_2S production indicates the presence of Escherichia, Klebsiella and Enterobacter, while those acid over acid reaction without the production of H_2S indicates the presence of Citrobacter, Arizona and Proteus.

Isolates from Triple Sugar Iron slant were stabbed to Lysine Iron Agar slant, all of which reacted positively, turning the agar into black because of the production of Hydrogen sulphide.

From Lysine Iron Agar, sample isolates were stabbed and streaked to Citrate agar, all of which showed positive reaction, converting the color from the original deep green to a deep blue. Citrate agar is a medium

used to determine whether or not the microorganisms have the ability to utilize citrate as sole source of Carbon. Salmonella sp. has the ability to utilize citrate. A positive reaction on Citrate Agar further verifies that the isolated organisms were Salmonella sp.

Morphological Characteristics and Gram Stain Reaction

Colonies of Salmonella sp. from Citrate agar were picked and fixed in a microscope slide for Gram Staining.

Microscopic examination using the oil immersion objective revealed typical Salmonella sp. characterized by rod-shaped morphology and pinkish to reddish cells indicating Gram negative bacteria.

CONCLUSION

Plastic chopping boards had a higher total bacterial count and presumptive Salmonella sp. compared to wood chopping boards. Results of ANOVA revealed significant differences in total microbial load and presumptive Salmonella sp. load between plastic chopping board but these were not influenced by time of sampling.

Colourless to greyish colonies in Mac Conkey plates were suspected Salmonella sp. and were subjected to selective media using Salmonella-Shigella agar. Isolates reacted positively showing blackening of some portion of the media indicating the reaction of the microorganisms to the ferric ions in the medium.

Suspected Salmonella sp. were picked, stabbed and streaked to Triple Sugar Iron, Lysine Iron Agar and Citrate Agar for confirmatory biochemical analysis. Sample isolates from TSI showed alkalinity on slants indicated by red color, acid production indicated by yellow butts, H_2S production by black colours on the slant and gas production by producing breaks on the slant. All isolates utilized the heavy elements on the LIA indicated by the change of color in the butt of the slant from purple to black color. Isolates on the Citrate agar utilized Citrate indicated by the change in color of the slant from green to deep blue verifying that they are Salmonella sp.

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