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Solitude and Delineation of Bacteria Associated with Hawked Pork-Meat

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Abstract
This project work was carried on the solitude and delineation of pathogenic bacteria from hawked Pork meat. Pork meat were bought from the sellers and was collected in raps of aluminum foil. It was then taken to the laboratory for analysis. The sample was homogenized then pipette using 5ml sterile pipette and test tube. The dilution was then cultured on different media, MacConkey agar, Nutrient agar and blood agar then incubated at 37^0C for 24 hours. Discrete bacteria colonies were observed and each colony was gram-stained and examined microscopically. Biochemical test was carried out to confirm the particular bacteria present. Bacteria isolated were Staphylococcus aureus 32.4%, Bacillus subtilis 51.9%, and Escherichia coli 15.6%. This microorganism isolated from contaminated Pork meat are capable of causing illness. For instance, Staphylococcu aureus produce air exo-toxin and can withstand temperature of 100^0C for 30 minutes, when ingested, it causes intoxication manifesting in 4 – 5 hours with acute diarrhea, vomiting and gastroenteritis lasting 6 – 8 hours.

Introduction

Meat is the flesh of animals which serves as food, it is obtained from sheep, cattle, goat and swine (Haman, 2002) meat is a major sources of protein and an important source of vitamins for most people in many parts of the world, thus they are essential for the growth, repair and maintenance of body cells which is necessary for our everyday activities.

Meat could be traced back to human history, when primitive men use raw flesh of dead animals. But as man develop, he domesticated wild animals. Beef have been the major supply of meat in Nigeria as a result of extensive and semi-intensive cattle production system in Nigeria by Fulani and Hausa people of the northern Nigeria (Umoh, 2004). Pork meat is a boneless lean meat of mutton, beef, goat or chicken meat staked on sticks, coated with sauces, oiled and then roasted over wood using fire from charcoal. It is a traditionally processed meat product and is usually not done with strict hygiene condition because they are still done locally. This is served hot and sold along streets, at clubs, picnics centers, and restaurants and within institutions. Pork meat is one of the intermediate moisture products that are easy to prepare and highly relished.

Materials and Method

Collection of Samples

Sample of Pork meat (400gm) was collected with a sterile container from ten different phase of preparation in Enugu metropolis then transported to the laboratory for proper examination.

Sterilization of Glass Ware

Already, properly washed petri dishes, test tubes, conical flasks, beaker, pipettes, spatulas, wire loop was wraped with crafting paper and sterilized on hot air oven at 160^0C for one hour and stored at 4^0C

Preparation of Sample

The samples were homogenized in 90ml of distilled water in a different beaker, this was centrifuged and the suppurate was collected and diluted in a tenfold serial dilution using normal saline as diligent. 10mls of homogenized samples were mixed with 90ml of the dilution prepared until a tenth tube which gave a final dilution of 10^{-10} incubation was done using the eightth, Nineth and tenth dilution (10^{-8} x 10^{-9} x 10^{-10}). This prevents the likelihood of overcrowding the plate with microorganism.

Preparation of Media

28g of nutrient agar powder was weighed and dissolved in 1 litre of deionise water then allowed to soak for 10 minutes swirl to mix then sterilize by autoclaving at 15b for 15 minutes at 121^0C cool to 47^0C. 52g of the commercial MacConkey agar powder was weighed and dissolved in 1 litre of ditstilled water in a conical flask and then autoclaved at 15bl pressure at a temperature of 121^0C for 15 minutes.

Blood agar was prepared by adding 25ml of blood to 500ml of already prepared nutrient agar at a temperature of 30^0C since above this temperature the blood will no longer give blood agar but chocolate agar.

Plating Technique (Spread Plate)

Aliquot of 0.1ml from the sample was spread on sterilized media in petridishes. These was spread using a hockey stick and thereafter incubated at room temperature for 24 hours.

The Streak Plate Method

Portion of each dilution was placed on the surface of an agar media and then streaked with a sterile wire loop. Actual inoculation was accomplished by streaking the agar surface with either the continuous streaking pattern or the two-phase streaking patterns. During the streaking process, care is taken not to cut into the agar medium. The wire loop was sterilized between each successive inoculation. The purpose of this techniques was to dilute the inoculation sufficiently on the surface of the agar so that isolated colonies of bacteria could be obtained from colony forming units. The bacteria cells were sufficiently far apart in some areas of the plate to ensure that the colony developing from one cell would not merge with that growing from another. The isolated colonies were individually sub cultured to another media to obtain pure culture of the isolate which were studied on different media.

The Pour Plate Method

The pour plate technique was done to dilute the specimen at 45^0C the medium was maintained in a liquid state at a temperature of 45^0C to permit a thorough distribution of innoculum within the medium. The inoculated medium was poured into Petri dish allowed to solidify and the plates were then inverted and incubated for 24 hours at room temperature for 37^0C. After incubation, the grown colonies on the plates were separately and individually sub-cultured onto fresh medium that support the growth of a particular specie of organism.

Staining Techniques Used Are Gram Stain

The specimen was collected from the growth of the organisms in the plate medium. it was smeared on a clean grease free slide then laid across a staining rack/staining dish and allowed to air dry. The heat fixed smear was flooded with 0.5% aqueous crystal violet for 30 seconds (which is the primary stain). Then drained off excess stain and washed off the residual stain on the smear with excess of Luyols iodine acting as a mordant. This means that the iodine increases the interaction between the cell and the primary stain for the cell to be stained strongly. Then allow the luyols iodine to act for 30 seconds. The smear was rinsed briefly with 50:50 acetone alcohol for 1 – 3 seconds for decolourization until blue colour ceases to come. This step generates the differential aspect of gram stain. At this stage, gram-positive bacteria retain the crystal violet while the gram-negative bacteria lose their crystal violet and become colourless. The smear was quickly washed with distilled water to avoid excess decolourization. The slide was laid across the staining rack again and the smear was flooded with 1% safranin to act for 60 seconds (1min) it was now washed off with distilled water. The smear was dry between folds of filter paper and further in air. Now the smear on the slide was examined under the x10 objective of the microscope. Gram positive stained dark blue while gram negative bacteria appeared pink red. Viable count techniques was used to know the average number of bacteria per sample in this technique, the serially diluted sample were incubated at 37^0C. It was assumed that each colony develop from one bacterium or viable unit only colony forming unit were counted. plates with discrete colonies between 30 – 300 were counted, less than 30 were few to count. The average bacteria count of the colonies was calculated per dilution by the reciprocal of the dilution factor.

Microbial Count

After incubation, the numbers of colonies on the Petri dishes were counted using the colony counting chamber. The average total and differential standard plate count (SPC) were taken.

Biochemical Test Use in The Identification of The Isolates

Catalese Test: A loopful of the test culture was placed on a drop of normal saline solution and smeared evenly on a clean slide. A drop of hydrogen peroxide was poured onto the smear and inspected for gas bubbles. Rapid effervescence indicated oxygen gas production and a positive test.

Coagulase Test: Two forms of coagulase occure free coagulase tube test and a slide test.
Slide Coagulase Test: This is used to differentiate staphylococcus clumping when a colony dropped on a plasma positive.

Motality Test

2 – 3 drops of peptone water with growth of the organisms was placed on a clean slide with the aid of a wire loop. The cover slip was placed over the slide. The slide was left for some time and then examined microscopically with the aids of high-power objective. Motile organisms would be seen swimming around.

Indole Test

The peptone water medium was inoculated and incubated for 48 hours at 37^0C the tube was further allowed to stay for another 48 hours in the incubator for the accumulation of indole after this period, 0.5ml Kovas was added separately to each tube and swirled gently. The appearance of a red colour in the alcohol layer indicated a positive reaction.

Sugar Fermentation Test

Sugar fermentation test were carried out using the following sugars glucose, mannitol, lactose and sucrose. To each 10ml of peptone water in a test tubes, 1.5g of each sugar was separately dissolved into and labeled. On each 3 drops of 0.01% phenol red was added. Durham tubes were inserted in an inverted position into the tubes for detection of gas production. The tubes were plugged with non-absorbent cotton wool and sealed with Aluminum foil then put in autoclave at 121^0C for 15mins at 15lb pressure. After sterilization, any trace of air in the Durham tubes were removed by inverting the test tubes. The tubes were then aseptically inoculated with small bacterial culture using sterile wire loop. The tubes were incubated for 24 hours at 37^0C and un inoculated tube served as controls.

Acid production was indicated by a change in colour from orange (alkaline) to yellow (acid) in the fluid. And gas production was indicated by the presence of air space at the bottom or sealed at the inverted durham tubes.

Result of The Food (Pork Meat) Analysis

Table 1: Plate count of colonies per ml

S/N	Plate Count
1	204
2	134
3	36
4	22
5	53
6	89
7	1
8	84
9	105
10	54
Total	782

Table 2: Standard plate count of each colonies isolated from each plate.

Sample Number	Designation of colonies		
	A	B	C
1	68	35	101
2	44	28	62

3	12	2	22
4	7	5	10
5	17	8	28
6	29	11	45
7	-	-	1
8	24	-	60
9	35	26	44
10	16	9	27
Total	**252**	**124**	**400**

Table 3: Percentage distribution of bacteria isolated

Code	**Mean Count**	**Percentage occurrences**	**% Distribution**
A	25	$\frac{25}{77} \text{ x } \frac{100}{1} = 32.4\%$	32.4%
B	12	$\frac{12}{77} \text{ x } \frac{100}{1} = 15.6\%$	15.6%
C	40	$\frac{40}{77} \text{ x } \frac{100}{1} = 51.9\%$	51.9%
	77		100%

Table 4: Identification of isolates on different media

	Morphological characteristics	**Gram reaction**	**Presumptive organisms**
A	Cream small round colonies on nutrient agar	Gram positive cocci	Staphylococcus spp
B	Smooth pinkinsh coloured colonies on MacConkey agar	Gram negative Rods	Escherichia coli
C	Large woody golden yellow colonies	Gram positive Rods	Bacillus spp

KEY

A = Cream small round colonies
B = Smooth pink colonies
C = Large woody Golden Yellow colonies

Table 5: Confirmatory result of the organisms isolated

	Motility	Citrate	Oxidase	Catalase	Coagulase	Indole	Glucose	Sucrose	Maltose	Manitol	Organisms
C	-	+	+	+	_	_	Ag	Ag	Ag	Ag	Bacillus subtilis
B	+	-	_	_	_	+	Ag	A	_	Ag	Escherichia coli
A	_	-	_	+	+	_	Ag	A	A	_	Staphylococcus aureus

The identification of bacteria presents in the Pork meat sample collected was based on their colonial characteristic gram staining reaction and on the biochemical test. Those bacteria isolated includes staphylococcus spp, Escherichia coli and Bacillus spp. The numbers of colonies of bacteria were counted by dividing the culture plate into four equal parts and each parts were counted by counting the single colonies in the plate. The number in each parts were added together to get the overall total numbers of the colonies present in each plates. The numbers of colonies of bacteria counted in each media are shown in the table above.

Conclusion

Staphylococcus are the major microbe that contaminates Pork meat therefore it is a common meat spoilage organism both before and after processes. On further sub culturing of the colony the presence of coliform in the meat sample was identified. Apart from being indicator organisms of facial contamination, certain strains of Bacillus and Escherichia coli or pathogenic or as Enteror pathogenic Escherichia coli having a serious effect on the victim after an incubation period of 7 – 12 hours with diarrhea, abdominal pain, headeache and vomiting. A selective media is recommended for that such as MacConkey agar to enable identify the colonies of the organism on the medium. It was observed at the point of collection that the seller may contaminate the food with saliva by excess talking also contamination could result from impure water used in washing the utensil and also the environment for which the food substance was produced if not hygienic.

Recommendation

Sellers should not mind the heating cost as they stand to reduce the rate of microbial load. Awareness as regard to hygiene should be created to the sellers to minimize the rate of contamination through carelessness. If Pork meat must be hawked, it should be carried about in glass case or ware that does not expose it to microorganisms as to reduce the incidence of food poisoning in our society. Quality control unit should be established in meat processing industries in Nigeria and hazard Analysis critical contol point (HACCP) concept should be applied to the processing and enderings of meat and Pork meat beef products, this will go a long way in reducing contamination and spoilage of meat products. Proper meat transportation, sanitation of utensils and equipment should all be employed to reduce microbial contamination.

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