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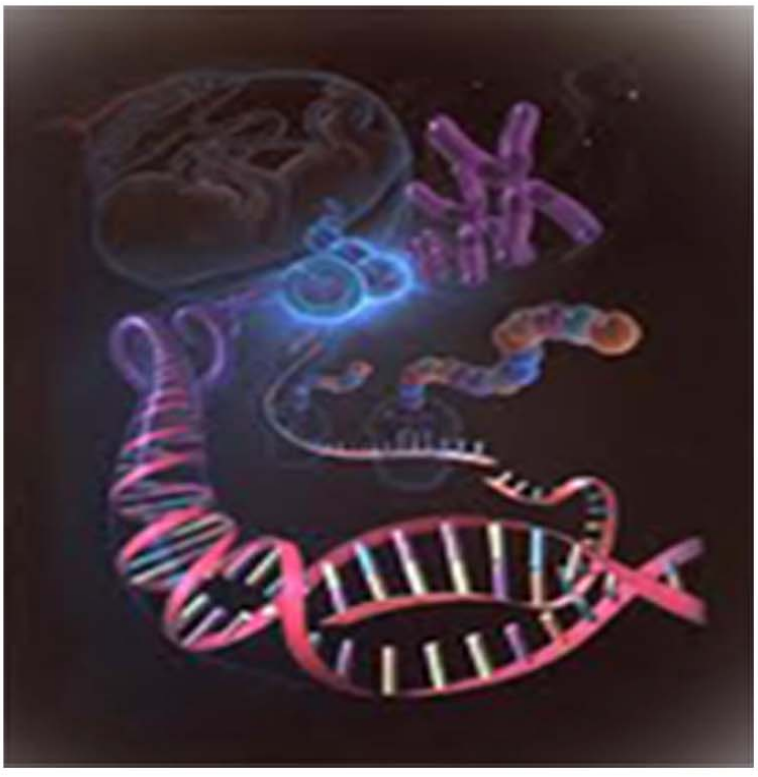

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Research Paper

BACTERIAL CONTAMINANTS ASSOCIATED WITH COMMERCIAL POULTRY FEEDS IN ENUGU NIGERIA

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Bacterial contaminants that are associated with commercial poultry feeds from three different companies in Enugu, Nigeria were studied. Poultry feeds which included starter, grower, finisher and layer were examined using pour plate techniques. The culture media used were CLED agar and *Salmonella* – Shigella Agar (SSA). The contaminants isolated include *Escherichia coli*, *Salmonella* spp and *Proteus* spp. *E. coli* was the predominant bacteria with 55 mean plate count and 42% followed by *Proteus* with 44 (33.6%) and *Salmonella* 32 (24.4%). The results showed that the poultry feeds in Enugu, Nigeria analyzed had bacteria contaminants.

Keywords: Bacterial contaminants, Feeds, Poultry, *Escherichia coli*, *Salmonella* spp., *Proteus* spp.

INTRODUCTION

The term 'poultry' used in agriculture generally refers to all domesticated birds kept for egg laying or meat production. Poultry comes from the French word *poul*, which was derived from Latin word *Pullus* meaning small animals. Poultry is the second most widely eaten meat in the world, accounting for about 38% of the world meat (Raloff, 2003).

The diseases of poultry is like the disease of other animals. They may be caused by pathogenic organisms, nutritional deficiency and from wound or cannibalism. Some of the diseases associated with fowls locally include; newcastle disease, chronic respiratory disease, fowl typhoid and fowl pox diseases.

Livestock (poultry) get infected when pathogenic organism passes to the susceptible animal through feeding (Barnes *et al*., 2003).

To prevent pathogenic organisms from getting into the body of poultry, attention should be given to the factors that influence their infectious spread. First and foremost, they should have disease-spreading stock, clean range, proper feeding and quarantining new stock. Sanitation is very important in poultry management by cleaning of their water can, feeding troughs and finally disinfecting the to help reduce organic matter.

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Poultry feeds are infected during processing, by handling, mixing of ingredients and exposing the raw materials and finished products to the atmospheric microorganism. Therefore, high rate of poultry disease and death occur as a result of consumption of contaminated feed and unpurified water.

Bacterial organisms affect the essential requirements of the body, such as water, carbohydrates, fats, vitamins, minerals and protein, thereby reducing the content of nutrients needed for the food to be palatable and easily digestible.

The aim of this research was to isolate and determine the contaminants that are associated with poultry feeds in Enugu, Nigeria.

MATERIALS AND METHODS

Autoclave, Incubator, Weighing balance, Bunsen balance, Wire loop, Hot air Oven, Cotton wool, Water bath, Spatula, Aluminium foil, Microscope, Blender, Beakers , Conical flask, Slides, cover slip, Petri dishes, Pipettes, Reagent bottles, Test tube and Calibrated cylinder.

Media Used

Cled agar and Salmonella -Shigella Agar (SSA)

Reagents

Crystal violet, Lugal's Iodine, Acetone Alcohol, Safranin, Methyl red, Kovac' reagent, Hydrogen peroxide and Peptone water.

STERILIZATION OF MATERIALS

The glassware and the wire loops were properly washed, air dried, wrapped with kraft paper and sterilized in hot air oven at 180^{o}C for 2 h.

COLLECTION OF SAMPLE

Poultry feeds consisting of ten lots were obtained from three different commercial feed-producing companies at Enugu, Nigeria. The samples (lots) for microbiological analysis were aseptically collected in sterile polythene bags and then taken to laboratory for analysis.

PREPARATION OF MEDIA USED

The media were aseptically prepared as when necessary according to the manufacturer's instructions on the labels of the media and autoclaved at 121^{o}C for 15 min.

MICROBIOLOGICAL ANALYSIS

1 g of each sample was dissolved thoroughly in 9 ml of distilled water and used to preparing five folds serial dilution. Using pour plate method, the media were inoculated. The mixtures were turned clockwise and anticlockwise to ensure even distribution, the pour plates were allowed to solidify and incubated in an inverted manner for 24 h. The plates were observed and the single colonies picked for sub-culturing in order to obtain pure culture.

MICROSCOPIC EXAMINATION

Microbial Count

After incubation, the number of colonies on the petri dish were counted using bacterial colony counting chambers. The average total and differential Standard Plate Counts (SPC) were taken.

Gram Staining Reaction

Firm (smear) of each of the isolates were prepared by picking a small portion of microbial growth from the plates with the aid of a sterilised

wire loops into a drop of sterile distilled water on glass slides and after making the smear, the slides were heat fixed by carefully passing them over a Bunsen burner flame.

The heat fixed smears were stained with crystal violet for 60 s, washed off with water and drained, then flooded with Lugol's iodine for about 60 s and washed off gently with water and drained. The samples/slides were rinsed with 50-50 alcohol – acetone for 3 s and were rinsed with water and drained. The slides were then counter stained with Safranin for 1 min (60 s) after then, the stains were washed off with water. The slides were air dried. Immersion oil was dropped on the smears and examined under the oil immersion objective of the microscope.

Biochemical Tests

Indole Test

5 ml of peptone water was incubated for 24 h at 37°C in test tubes. The isolates were grown into the peptone water and allowed to stay for 24 h. After, 5 drops of Kovac's reagent were added separately on each test tube and swirled gently for 5 min. Positive reactions were indicated by the development of a red color in the reagent layer above while in the negative reaction (result) the indole reagent retained its yellow color.

Methyl Red Test

The isolates were grown in 5 ml of MR both (glucose – phosphate peptone water) and incubated for 48-72 h at 37°C. Thereafter, 3 drops of methyl red were added into each test tube. A reddish colour on the addition of indicator signified a positive result while a yellowish color denoted negative result.

Voges – Proskayes Test

Isolates were grown in 5 ml of Peptone water and glucose, respectively. This was incubated for 48-72 h at 37°C then 5 drops of potassium hydroxide (KOH) was added. The tubes were shaken at intervals to ensure maximum aeration after 2-5 min. The development of red color within 30 s and 60 s indicated a Voges-Proskauer positive test. But no red color was seen which showed a VP negative result.

Catalase Test

A small amount of the culture organism was picked from agar plates using a clean sterile wire loop. This was tented in drops of hydrogen peroxide (H_2O_2) on a clean microscopic slide. Production of gas bubbles indicated a positive reaction while absence of bubble indicated a negative reaction.

Motility Test

About 2-3 drops of peptone water was dropped on a clean slide and isolates introduced on the slides with the aid of wire loop. Cover slips were placed over each of the slides. The slides were left for some time and then examined microscopically with the high power objective. Motile organism swimming around indicated positive reaction while non- motile organize indicates a negative reaction.

Oxidase Test

A piece of filter paper was wetted with a few drops of 1% oxidase reagent solution (tetraethyl-phenylene dihydrochloride). A bit of the isolates were obtained with a sterile wire loop and smeared on the wetted portion of the filter paper. The development of an intense purple color within 30 s indicated a negative test.

Carbohydrate Fermentation by Triple Sugar Iron Agar Test (TSI)

In this test, 10 ml of peptone water was introduced into each of 3 sterile test tubes. 1 g of carbohydrate such as glucose, lactose and sucrose were added into each of the test tubes, respectively and labelled accordingly. They were stirred to dissolve completely over a Bunsen burner. After which 3 drops of methyl red were added into each of the test-tubes which served as an indicator and a base medium. The tubes were then plugged with cotton wool and sterilized at 110^{o}C for 15 min. They were then incubated at 37^{o}C for 24 h. A change in coloration of the medium after 24 h from purple to yellow indicated acid production due to the fermentation of the sugar by the organism while retention of the purple color indicated a negative reaction. Gas production was shown by the present of gas bubbles on the surface of the medium and an up word movement of the inverted durham's tube and the result was noted and recorded.

RESULTS AND DISCUSSION

This study was conducted in order to determine and investigate the bacterial contaminants of poultry feeds. Four types of poultry feeds consisting of ten samples were examined using different media. Table 1 represents the total plate count of bacteria for all of the samples on the selective media. Table 2 represents the standard plate count of the different colonies formed. Here, counting is based on the colors of the colonies formed on CLED media, the alphabet A, B and C in the table represents different colors on the CLED media that is the designation of colonies of the samples.

Table 3 represents morphological characteristics of the bacteria isolates. The bacteria were identified by determining their colony morphology in the selective media (CLED and SSA). Poultry feeds were contaminated with *Escherichia coli* and *Samonella* spp. (Parker, 1990). Among the

Table 1: Total Plate Count Of Bacterial Isolates from Poultry Feed

S. No.	Plate Count
1.	140
2.	122
3.	125
4.	138
5.	128
6.	136
7.	137
8.	129
9.	130
10.	124
Average	131

Table 2: Standard Plate Count of Different Colonies Formed

No. of Sample	A	B	C
1	38	22	80
2	30	22	80
3	52	41	32
4	62	46	30
5	49	38	41
6	56	45	35
7	70	–	66
8	82	47	–
9	50	28	52
10	56	33	35
Average	55	32	44

KEY: A= *E.coli*, B = *Salmonella* and C= *Proteus*

Table 3: Morphological Characteristics of Bacterial isolates from

Morphological Characteristics	Gram Reaction	Presumptive Organisms
Deep yellow, round coloured colonies on cled agar	Gram –ve rod in pairor single	*E. coli*
Brown round colonies on SS Agar	Gram –ve rod in single	*Salmonella Spp*
Small blue coloured colonies on cled Agar	Gram –ve rod in pair	*Proteus Spp*

Table 4: Biochemical Tests for Isolated Organisms

Indole Test	Methyl red Test	Voges-proskagyes	Motility Test	Catalase Test	Oxidase Test	Glucose	Lactose	Sucrose	Mannitol
+	+	+	+	-	-	AG	AG	G	AG
-	+	+	+	-	-	AG	G	G	-
-	-	-	+	-	-	A	-	-	A

Identified organisms
Escherichia coli
Salmonella spp.
Proteus spp.

KEY: – =Negative; G = Gas; + = Positive; AG = Acid and Gas; A = Acid.

Table 5: Percentage Distribution of Bacteria Isolated

Bacteria	Mean Plate Count	Percentage Distribution
E coli	55	42%
Salmonella	32	24.4%
Proteus	44	33.6%

bacteria, *E. coli, Samonella* spp. and *Proteus* strains were isolated from poultry feeds. Colony morphologies in the selective media determined identified bacteria.

Table 4 represents the biochemical tests for the isolated organisms. From the gram's staining reaction, the bacteria isolated and identified were gram negative bacteria. Finally Table 5 represents the percentage distribution of isolated bacteria from the feed sample. *E. coil* had the highest percentage distribution, followed by *proteus spp* and *Salmonella* spp.

CONCLUSION

In conclusion, *E. coli, Salmonella* spp. and *Proteus* were the major poultry feed contaminants in Nigeria.

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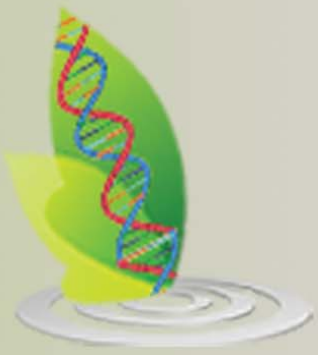

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