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Phenotypic characterization of food borne bacteria isolated from different chicken organs in Al- Sharkia Governorate, Egypt

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ABSTRACT : This study was conducted to isolate and identify the possible pathogenic bacteria from different chicken organs in Al-sharkia government using biochemical and standard culture procedures. A total of 100 chicken organs samples (Breast , Liver , Drumstick , Wings , Skin , Intestine , Pins, Giblets , Heart and Legs) from local chicken were collected during a period from November 2021 and May 2022. The bacteria were diagnosed according to morphological, cultural and biochemical characteristics. Bacteriological examination of the samples showed both Gram-positive and Gram-negative bacteria in Breast (n=22) , Drumstick (n=18) , Liver (n=11) , Wings(n=12) , Skin (n=4) , Intestine (n=12) , Pins (n=8) , Giblets (n=12) , Heart (n=8) , Legs (n=5) with isolation rates of 19.6% , 16.1% , 9.82% , 10.7% , 3.6% , 10.7% , 7.14% , 10.7% , 7.14% , 4.5 % as well as to examined the phenotypic and genetic relationships between bacterial isolates from the different chicken organs that were resistant to critically important antibiotics. Bacillus species that were also examined for their biochemical character.

KEYWORDS: Isolation, Diagnosis, Bacteria, Chicken organs, phenotypic

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I.   INTRODUCTION

A major worldwide health issue affecting both humans and animals, is antimicrobial resistance (AMR) more prevalent and severe in low- and middle-income nations than in high-income ones (Laxminarayan, *et al.*2016; Hedman, *et al*. 2020). A problem in resource-poor countries, illogical, indiscriminate, and improper use of antibiotics in humans, veterinary medicine, and agriculture are all associated to the formation of AMR. 1.3

million of the over 5 million global human deaths linked to AMR can be directly linked to bacterial **.** Due to its high nutritional value of proteins, amino acids, and vitamins all of which humans need chicken meat is currently one of the most popular meats consumed. It is also considered a healthy meat and is consumed by people trying to control their weight and prevent obesity because it is easy to digest and suitable for people of all ages (Al-Aswad, 1989).

Chicken meat that has not been contaminated is devoid of bacteria, but contamination happens quickly during slaughter, processing, and marketing due to carelessness or contamination from outside sources, such as contaminated knives, workers' hands, or the ground (Wilson and Sande, 2001). Because of the high rate of contamination with diseased chicken feces during the operations of slaughtering in slaughterhouses or butcher shops, poultry meat is one of the major sources that is accountable for infecting humans with many bacteria, including *Salmonella*, Staphylococcus, Bacillus and Escherichia coli (Chambers *et al* 1998) & (Baydvlato and Bscarthies 1984)& (Khammas and Abdullah 2015) (Hussein and AL-Attar, 201)
The aim of this study to identify some of the pathogenic bacteria present in different chicken organs from six distinct in Al-sharkia government

II. MATERIAL AND METHOD

2.1. Samples collection

During a period from November 2021 and May 2022 a total in Al-sharkia were used to collect a total of one hundred (100) different chicken organs randomly. one hundred grams of chicken samples were procured and put into sterile, dry, and clean polythene bags under cooling conditions before being delivered to the lab for microbiological examination and diagnosis.

2.2. Sample culturing

With a sterile knife, with ethanol 70% the samples were aseptically divided into small, thin pieces. Two hundred fifty mL of distilled water was used as a stock after the analytical parts were homogenized in separate sterile plastic bags. For the serial dilution experiment, 1 mL of stock homogenate and 9 mL of sterile distilled water were used to achieve serial dilutions up to five fold (10^{-5}) for each prepared sample. In order to obtain a distinct colony, this was done. We used the spread plate approach to pour the pre-serially diluted samples (0.1 mL) onto ready-made solidified nutrient agar plates. Mixture was leaved for 5 minutes before inoculation of plates which were then incubated at 37°C for 24 hours.

2.3. Identification of colonies via microscopy:

On the agar plate, the isolates' cultural traits such as size, pigment, margin, elevation, form, and surface were recorded beside the colour-results of the stain-reaction including Gram′s stain-test, capsule stain, and the motility test of the isolated bacteria, after subculture on the medium of the Nutrient broth at 37°C for 4 hours . **(Brown, 2005).**

2.4. Biochemical tests

 Biochemical tests were applied on bacterial isolates, which included gram staining, catalase, nitrate reduction, motility,urease, indole, methyl red, spore forming (**Forbes** *et al* **2007**)

2.5. Gram's Reaction

On clean, grease-free slides, the samples of the isolates were smeared and heat-fixed. Following the addition of Gram's iodine for 60 seconds, two drops of Crystal violet were added. The slides were water cleaned, then decolored with alcohol for 15 seconds before being water rinsed again. The decolorized slide was counter stained with Safranine for 60 seconds. It was rinsed off using water. The slides were air-dried and viewed under the microscope using oil immersion lens (Forbes et al 2007).

2.6. Analyze catalase:

A few drops of 3% H2O2 regent were immediately applied to the bacterial growth on the agar plate culture. Catalase-producing organisms are discovered using this assay. By dissolving hydrogen peroxide into water and oxygen gas, this enzyme detoxifies it. The presence of oxygen gas bubbles obviously indicates the presence of catalase (Lelliott and Stead, 1987).

2.7. Nitrate reduction:

Durham's tubes were placed upside down, 0.1% KNO3 was dissolved in the broth, and they were sterilized for 5 drops of reagent A Sulfanilic acid solution and 5 drops of reagent B N,N-Dimethyl-1-naphthylamine should be added to the test tube holding the culture. Shake the tube vigorously to mix the chemicals and the medium. The appearance of a characteristic red or pink hue, which should occur within a few minutes, can be used to identify nitrate reduction. (Stanier et al., 1966)

2.8. Urease test:

Without being stabbed, the Christensen's medium's slant was heavily inoculated with the test bacterial sample. The contaminated tubes were kept in an incubator for 24 hours at 37 °C. Hydrolysis is the process by which the ammonia splits, turning the red colour of urea from its purple pink hue. (Arora, 2003).

2.9. Methyl red:

The bacterium was inoculated onto glucose phosphate peptone after a 24-hour incubation period at 37°C. Very little of the methyl red indicator was used. The hue yellow represented a successful test (Koneman et al., 1997).

2.10. Gas production:

The generation of carbon dioxide gas from glucose was crucial in identifying the isolate's homo-hetero fermentative nature. Inverted Durham tubes containing 10 mL of phenol red glucose broth and pure culture isolates were incubated at 37° C for 48 hours (Koneman et al., 1997)..

2.11. Indole test:

The isolated bacteria were inoculated into peptone water and incubated at 37°C for 24 h. Then 5 drops of Kovac's reagent were drowning on the inner wall of the tubes. A deep red ring was indicate. Positive reaction and negative one revealed yellow color at the alcoholic layer (Arora, 2003)

III. RESULTS

3.1. Distribution of the collected isolates:

The results of the distribution percentage and number of bacteria isolated from each chicken organ indicated that the maximum number of the isolated bacteria were collected from Breast (19.6%) and Drumstick (16.1%) samples. However, the minimum number were presented in skin (3.6%) and legs (4.5%). The other sources of isolated samples showed an oscillatory distribution ($p<0.001$; Table 1 and Figure 1)

Table 1: Distribution percentage and number of bacteria isolated from each chicken organs

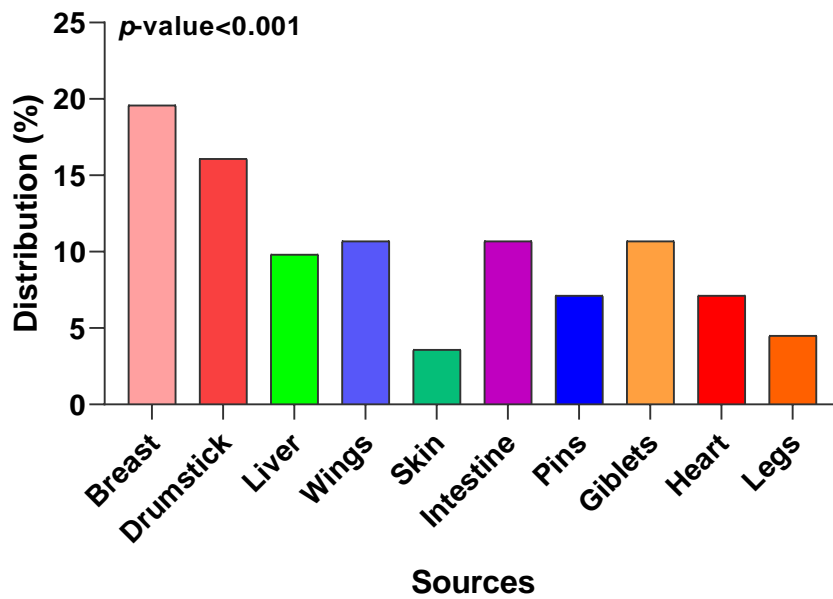| Source	Bacterial isolates	
	No.	Distribution (%)
Breast	22	19.6
Drumstick	18	16.1
Liver	11	9.82
Wings	12	10.7
Skin	4	3.6
Intestine	12	10.7
Pins	8	7.14
Giblets	12	10.7
Heart	8	7.14
Legs	5	4.5
Total isolate number	112	100
p-value	<0.001	

Figure 1: Distribution percentage and number of bacteria isolated from each chicken organ.
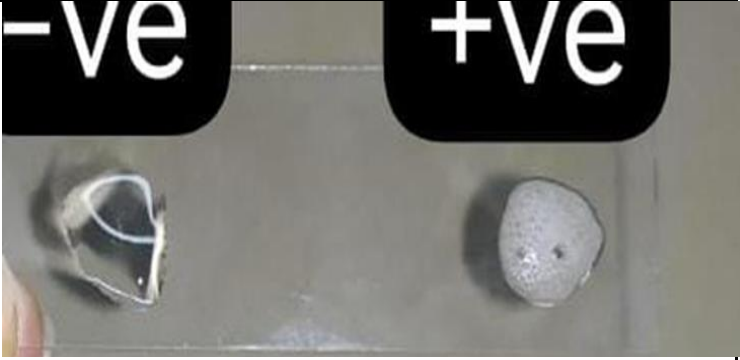
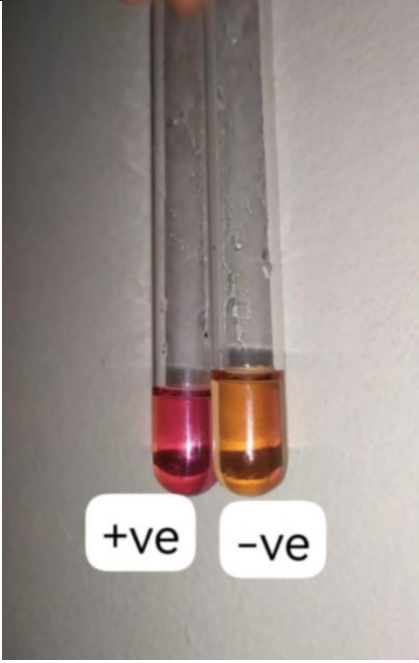### 3.2. Characterization of isolated strain's phenotype

Bacillus strains from various origins (Table 1) exhibited phenotypic characteristics that were shown in Table 2). Urease, indole, methyl red, and gas generation had negative effects in contrast to catalase, nitrate reduction, motility, spore, and Gram stain's good positive effects. This biochemical test partially identified our isolate.
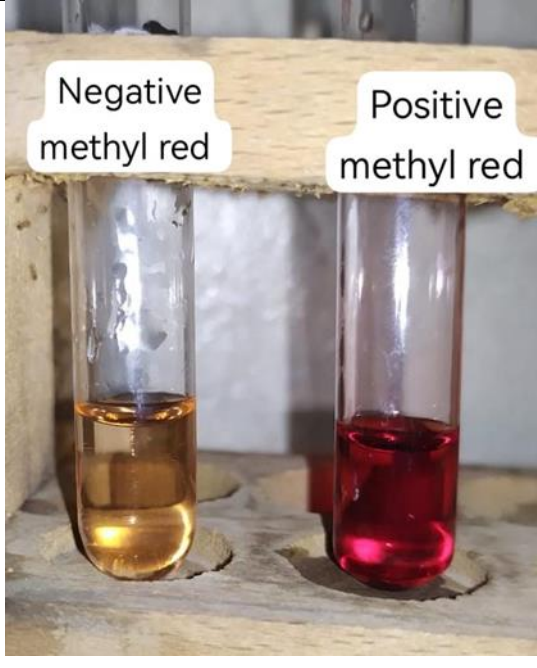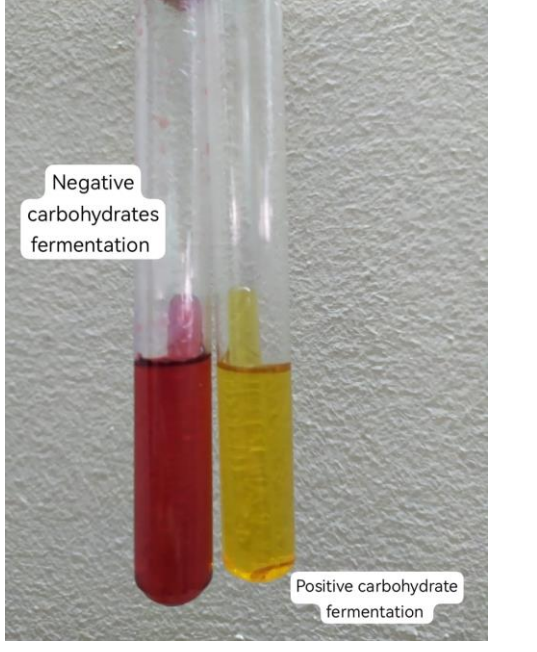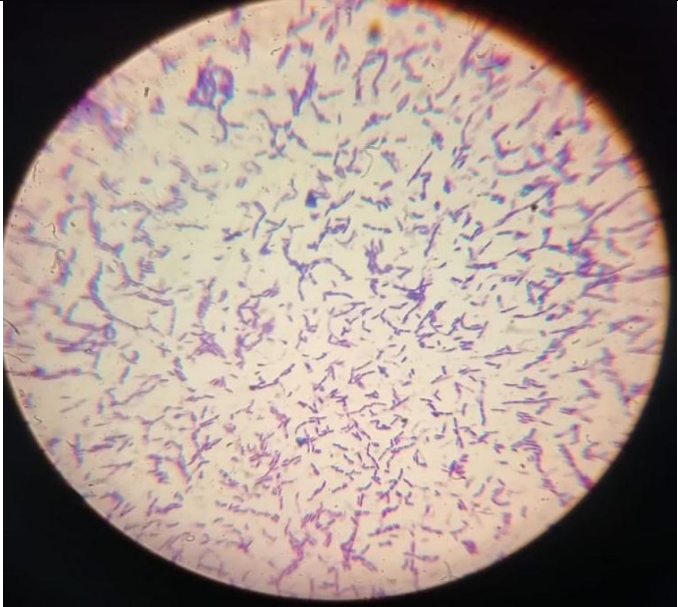

Figure 2: bacterial culture observation on nutrient agar medium after 24h of incubation

Table2: Growth and substrate utilization characteristics of isolated strain observed in this study.

Biochemical test	Result	Observation
Catalase	Positive	
Nitrate Reduction	Positive	

| Urease | Negative | |
| Indole | Negative | |

Methyl red	Negative	
Gas production	Negative	

Gram stain	Positive	
Spore	Positive	

IV. DISCUSSION

The current investigation's objective was to characterise the varieties and geographic distribution of bacterial pathogens. According to Public Health England (2009), less than 103 cfu/g or ml of bacillus group bacteria in food is considered to be appropriate. The results in Table 1 show that Breast has a significant proportion of bacillus occurrence. However, concentrations as low as 103 B. cereus cfu/g or m1 of food sample have been shown to cause food poisoning (Gilbert and Kramer, 1986; Stenfors Arnesen et al., 2008).

Due to there are two main reasons why poultry meat is frequently contaminated with enteric bacteria. The intense raising method that fosters rapid pathogen transmission among flocks and the extremely high throughput rates at major processing plants that facilitate the spread of germs among carcasses during processing may be the causes (Robinson et al., 2016).

Numerous food types contain the common pathogen Bacillus, and according to Arora (2003), it's crucial to remember that food products can quickly get contaminated owing to improper handling, storage, or equipment cleaning and sanitization methods. The bacterial burden may increase quickly and reach a harmful level of bacteria (up to 10^4 Cfu/g or ml) **(Public Health England, 2009)**

Bacillus species are essential parts of the bacterial ecosystems found in fresh chicken food because of the wide range of physiological adaptations they possess. The isolation and characterization of Bacillus species from various habitats will lead to the discovery of a variety of metabolic processes, the production of chemicals with biotechnological value such as enzymes and antimicrobial agents, and the presence of potential toxigenic components **(Tewari and Swaid, 2014)**. Biochemical tests enabled the accurate identification of Bacillus in our analysis.

Conclusions

From this results found the most prevalence bacteria found in poultry food is *Bacillus* species that were detected by biochemical and microscopic identification. So for further study investigate Bacillus by identification for elucidate from food poultry

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