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Research Paper

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Prevalence and phenotypic characterization of some pathogenic bacteria isolated from different meat sources in Al-Sharkia Governorate, Egypt

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Abstract: This study discussed the isolation and identification of some multidrug resistant bacteria that causing morbidity and mortality. (104) bacterial isolates were resulted from 100 samples of meat and meat products that were randomly collected from six different locations in Al-Sharkia Governorate, Egypt. These bacterial isolates were obtained from 10 different meat samples with the following percentage representations: fresh meat (6.73%), frozen meat (13.46) Brazilian meat (12.5%), Sudanese meat (4.81%) Pastrami (6.73%), Frozen burger (10.58%), Frozen kofta (14.42%) Minced meat (5.77%), Luncheon (18.27), and Sausage (6.73). The high percent of isolates present in Luncheon.

In the current studies both of phenotypic characterization and the identification of a few virulence genes were studied. The most potent strain fully characterized by biochemical characterization is *Bacillus spps.* transmission of multidrug-resistance STEC strains are linked with a severity of disease outcomes and horizontal spread of resistance genes in other pathogens. The result of this has emerged as a significant threat to public health, animal health, food safety, and the environment.

***KEYWORDS*: Multi drug resistant (MDR), *Bacillus*, and phenotypic.**

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I. INTRODUCTION

Considering meat contain a lot of moisture, a lot of nitrogenous chemicals (amino acids, peptides, and proteins), and a lot of minerals and auxiliary development factors, meat and meat products are perfect for many organisms to grow in tissue. Moreover, they maintain a pH that is ideal for the growth of the majority of bacteria and have some fermentable carbohydrates, typically glycogen (Mediani, *et al* 2022). Food-borne disease, often known as food poisoning, is any ailment brought on by ingesting contaminated food or beverages. Pathogenic bacteria, viruses, parasites, as well as synthetic or natural toxins derived from lethal mushrooms, contaminate food and cause this disease . (Abeyta, 1983).

Food-borne illnesses are a serious public health issue that has a high global morbidity and mortality rate. Infectious diarrhea causes 3-5 billion cases worldwide and up to 1.8 million fatalities a year, mostly in young children. (Adams and Moss, 1999) Animal flesh from the mammalian species is known as meat and is utilized as nourishment for humans. Skeletal muscles and their associated fat are referred to as meat, but it can also refer to other edible parts including organs, livers, skin, brains, bone marrow, kidneys, or lungs. Although meat is typically eaten cooked, many traditional recipes call for eating it raw or only partially cooked.

In recent years, the health benefits of meat as a regular part of the human diet may be offset by risks. Like any product, meat has the potential to spread some infections, although this risk is reduced by thorough cooking and avoiding cross-contamination. Trichinosis or cysticercosis-causing parasites can occasionally be found in undercooked pork. If the required procedures (such as steam pasteurization or organic acid treatment) are not implemented, minced beef can get contaminated during slaughter with disease-causing *Escherichia coli* O157:H7 originating from the digestive system or hide. (Ikeme, 1990). It has been noted that while the animal's exterior and digestive system are the main sources of bacteria during slaughter, dressing, and cutting, other sources include blades, clothes, the air, workers, carts, crates, and equipment in general. Since a wide range of different organisms are added, it can be assumed that under normal circumstances, the majority of potential spoilage organisms are present and will be able to grow if the right circumstances arise (Handare *et al* 2007). The goal of the current investigation is to learn more about the microorganisms that may be present in market-sold beef

II. MATERIALS AND METHODS

2.1. Collection of Samples

A total of one hundred (100) different samples of meat and meat products were randomly obtained from 6 different locations in Al-Sharkia Governorate. 100 g of meat samples were collected into clean, dry and sterile polythene bags and transported to the laboratory for microbiological analysis.

2.2. Culture Conditions for Isolation of Bacteria

The samples were aseptically cut into thin smaller pieces using sterile knife. The analytical portions were placed in separate sterile plastic bags and homogenized in 250 mL of distilled water, this was used as stock. Serial dilutions were achieved up to five fold (10^{-5}) for each prepared sample using 1 mL from stock homogenate and 9 mL of sterile distilled water for the serial dilution experiment. This was carried out in order to obtain discrete colony. We took pre-serially diluted samples (0.1 mL) and dispensed onto prepared solidified nutrient agar plates by spread plate method. This was allowed to set firmly for five (5) minutes afterward inoculated plates were incubated at 37^{o}C for 24 hours. Bacteria colonies observed after 24 hours were then sub-cultured into freshly prepared Nutrient agar in sterile petri dishes to obtain discrete (**Oluwatobi, *et al* 2021)** colonies and were subsequently identified.

2.3. Microscopic examinations

Microscopic examinations were carried out on the isolates for studying the shape, arrangement and reaction of the staining, which included Gram's stain, capsule stain, and the motility test of the isolated bacteria after subculture on the medium of the Nutrient broth at 37^{0}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, isolates were created as smears 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 Bartholomew and Mittwer (1952).

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, KNO3 was dissolved in the broth, and they were sterilized for 20 minutes at 115°C.(Stanier et al., 1966)

2.8.Urease test:

Without being stabbed, the Christensen's medium's slope was heavily inoculated with the test bacterium. 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 an isolate's homo-hetero fermentative nature. Inverted Durham tubes containing 10 Ml of nutrient broth and pure culture isolates were incubated at 37° C for 48 hours **(Goyal *et al*., 2012)**

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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 positive reaction and negative one revealed yellow color at the alcoholic layer (**Arora, 2003**).

III. RESULTS AND DISCUSSION

3.1. Prevalence of pathogenic bacteria in meat Samples

The maximum isolated bacteria were picked out from luncheon (18.27%). However, the minimum isolated were presented in Sudanese meat (4.81%). The other sources of isolated samples showed a wobbly distribution ($p<0.001$; Table 1 and Figure 1). Several types of foods include the common pathogen Bacillus species

according to the (**Adel *et al* 2023)**; the allowed level of bacteria in food is below 10^3 cfu/g or ml. Nevertheless, food poisoning may be brought on by levels as low as 10^3 bacteria Cfu/g or m1 of food sample (**Anita and Swaid 2015**). In reality, it's crucial to remember that food products could become readily contaminated as a result of improper handling, storage, or equipment cleaning and sanitization practices. As a result, the bacterial load could increase quickly and get to the harmful level (up to 10^4 Cfu/g or ml) (**David *et al* 2012)**.

Table 1: Distribution percentage and number of bacteria isolated from each meat source.

Sources	Bacterial isolates	
	No.	Distribution (%)
Fresh meat (M)	**7**	**6.73**
Frozen meat (F)	**14**	**13.46**
Brazilian meat (BM)	**13**	**12.5**
Sudanese meat (SM)	**5**	**4.81**
Pastrami (P)	**7**	**6.73**
Frozen burger (B)	**11**	**10.58**
Frozen kofta (K)	**15**	**14.42**
Minced meat (MM)	**6**	**5.77**
Luncheon (LC)	**19**	**18.27**
Sausage (S)	**7**	**6.73**
Total bacterial number	**104**	**100**
***p*-value**	**<0.001**	

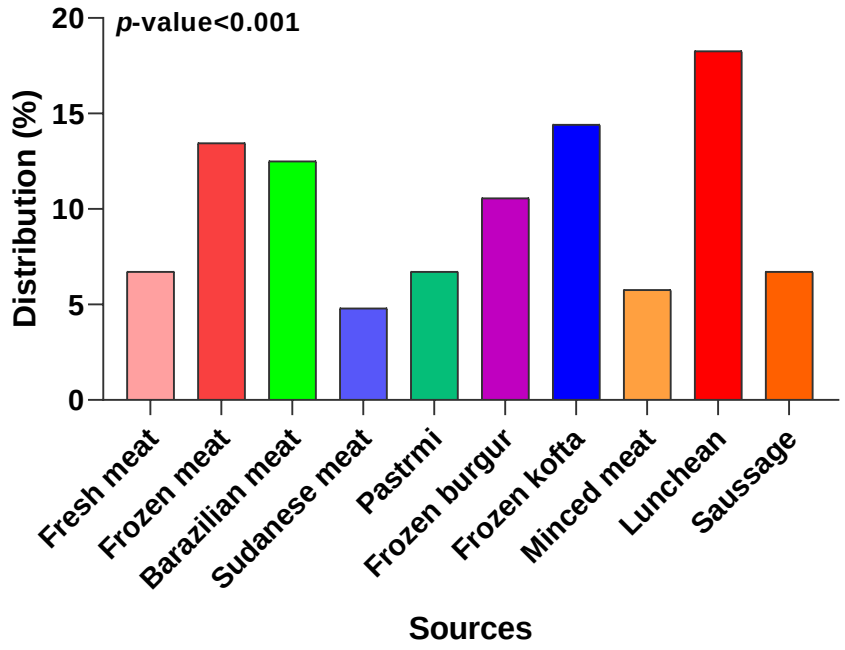


Figure 1: Distribution percentage and number of bacteria isolated from each meat source.

3.2. Phenotypic characterization of isolated strain

Strains obtained from various sources (Table 1) displayed consistent phenotypic traits (Table 2). In contrast to Catalase, Nitrate Reduction, and Gram stain give positive effect urease, Indole, Methyl red give negative effect. *Bacillus* genus was the prevalent species, according to this study on the diversity of *Bacillus* spp. detected by biochemical tests. *Bacillus* spp is a member of the aerobic spore-forming *B. subtilis* group, which has recently attracted a lot of study interest due to its role in cases of food poisoning (**Marwa and Bakri 2023)**.

Bacillus spp. is essential members of fresh meat food bacterial ecosystems because of their wide range of adaptable physiological characteristics. The isolation and characterization of Bacillus species from these

environments will aid in the discovery of novel mechanisms for environmental survival, a variety of metabolic processes, the production of biotechnologically useful substances like enzymes and antimicrobial agents, and the presence of putative toxigenic elements.

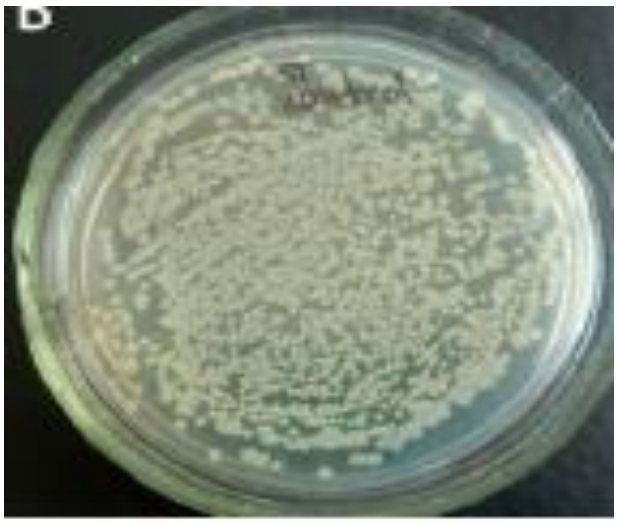
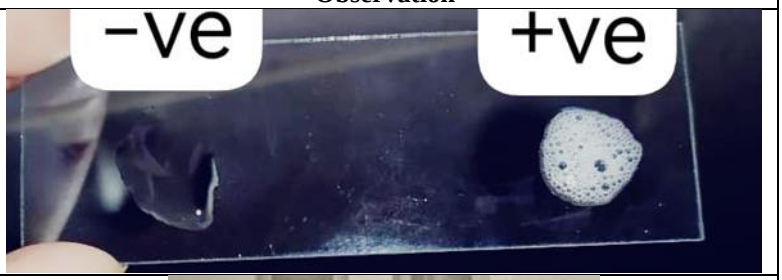
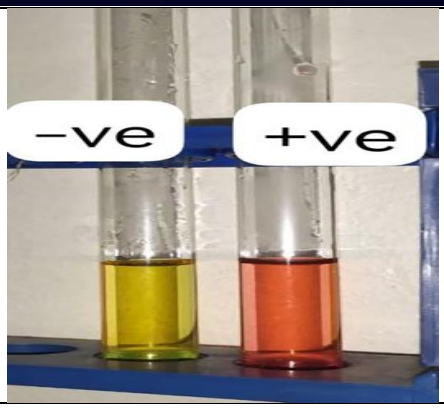
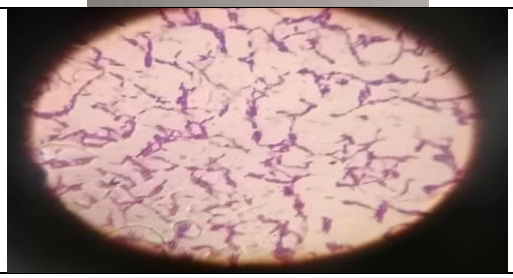


Figure 2: Bacterial cultivability after24 h of incubation **at 37 C^0**

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

Biochemical test	Result	Observation
Catalase	**+Ve**	
Nitrate Reduction	**+Ve**	

Urease	**-Ve**	
Indole	**-Ve**	
Methyl red	**-Ve**	
Gram stain	**+Ve**	

Conclusion:

From these results found the most prevalence bacteria found in meat samples is *bacillus* that was detected by biochemical and microscopic identification.

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