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Research Paper

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Prevalence and Antimicrobial Resistance of *Vibrio* Species Isolated from Beef Frozen Meat

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Abstract: *Vibrio* species can cause food borne infections and serious gastrointestinal illnesses. In this research, twenty samples of beef frozen meat were randomly collected from different markets in Zagazig, Sharkia Governorate, Egypt. Samples were subjected to microbiological analysis. All twenty samples were positive for *Vibrio*. The prevalence rate was 55% (11) for *V. mimicus*, 25% (5) for *V. vulnificus*, 20 % (4) for *V. parahaemolyticus*,20 % (4) for V. *fluvialis*, 10% (2) for V. *alginolyticus*,10% (2) for *V. cholera* and 5 % (1) for *V. hollisae*. All Vibrio was detected in all twenty samples. *Vibrio* strains demonstrated susceptibility to Ciprofloxacin (CIP) (100%), tetracycline (TE), Doxycycline (DO), Meropenem (MEM), Imipenem (IPM) and Chloramphenicol (C). While most vibrio strains were resistant to Amoxicillin/ clavulanic acid (AMC) (90.9 %) and Ampicillin (AM) (72.7%). In summary, this study provided a comprehensive overview of the incidence and phenotypic characteristics of various vibrio serovars found in beef frozen meat.

KEYWORDS: **Antimicrobial susceptibility, prevalence, *Vibrio*, beef frozen meat.**

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I.INTRODUCTION

Vibrios are small, motile rods that are gram negative, pleomorphic (curved or straight), or comma shaped. They have polar flagella that are sheathed, and their size is around 2 X 0.5µM. The majority of the *Vibrio* species in the Vibrionaceae family are facultatively anaerobic, halophilic, and release enterotoxins into food **(Nishibuchi and DePaola, 2005).**

A variety of virulence factors encoded by virulence genes contribute to *Vibrio* strain pathogenicity **(Schroeder *et al.*, 2017)**. In general, virulence factors allow pathogens to infect and damage the host by facilitating pathogenic adhesion and entry, establishment and multiplication, avoidance of host defenses, host damage, and finally get out from the infected host **(Darshanee Ruwandeepika *et al.*, 2012)**. *Vibrios* possess five important virulence factors: capsular polysaccharides, adhesive factors, cytotoxins, lipopolysaccharides, and flagella.

According to **Quadri *et al.*, (2005)**, pathogenic *V. parahaemolyticus* bacteria primarily cause gastrointestinal conditions that are characterized by watery diarrhea, nausea, and abdominal cramps. However, in extreme cases, septicemia and generalized disease may occur, which may cause death. It is well recognized that *Vibrio cholerae* causes asasiatic cholera, often known as epidemic cholera, a potentially fatal form of secretory diarrhea **(Faruque and Nair, 2008)**.

Along with being the main virulence factor for the cholera disease, *V. cholerae* also produces a variety of extracellular enzymes, such as lipases, nucleases, proteases, and chitinase, which is known as cholera enterotoxin (CT). Though *Vibrio cholera* is mainly a watery illness, this study distinguishes it from other foodborne pathogens that are also zoonotic. A number of food-borne illnesses, including cholera, gastroenteritis, open wound infections that result in septicemia, diarrheagenic cholera, etc., are caused by consuming frozen meat contaminated with invasive *Vibrio cholerae*.

Some studies reported that undercooking, using untreated night soil, washing with unclean water, and careless handling can all lead to the contamination of meat and meat products with *Vibrio* species **(Feachem *et al.*, 1981).** Although both raw and cooked meat can be infected, the risk of illness from consuming pathogens that exist in raw meat is much higher **(Rabbani and Greenough, 1999)**.

Meat can be improperly or insufficiently cooked, allowing disease-carrying pathogens to be ingested. Furthermore, meat can be infected at any stage of the production process, from slicing prepared meats to cross-contamination in a refrigerator. All of these conditions may increase the risk of the disease **(Azwai *et al.*, 2016)**. For exporting frozen beef meat to other countries, the statutory inspection system and standards should be rigorous. So, the aim of this study was to investigate the incidence of *Vibrio* spp in raw beef frozen meat as well as the effect of some antibiotics on *Vibrio* isolates.

II. MATERIALS AND METHODS

2.1. Sampling

A total of twenty samples of raw beef frozen meat were randomly obtained from different markets in Zagazig, at Sharkia Governorate, Egypt. Each sample 250g in weight, the raw meat samples were packed in sterile polyethylene bags. All the collected samples were labeled with the name of the market and date of collection and were transferred within two hours to the laboratory for thorough microbiological analysis.

2.2. Isolation and Identification

2.2.1. Isolation of *Vibrio* species*:*

10 gm from each meat sample was added into 90ml of alkaline Peptone Water (APW, HIMEDIA, M618-500G), Homogenized the mixture in a blender and then incubated at 37°C overnight. A loopful from the pre-enriched broth was streaked onto Thiosulphate Citrate bile-salt Sucrose agar (TCBS, HIMEDIA, M186-500G) plates and incubated at 37°C for 24 h.

2.2.2. Purification of Vibratos isolates

Typical colonies of *V. mimicus*, *V. parahaemolyticus*, and *V. vulnificus* showed smooth and green (sucrose negative), whereas colonies of *V. cholerae*, *V. alginolyticus*, and *V. fluvialis* appeared smooth and yellow **(Ibrahim *et al.*, 2018)** on TCBS agar plates were picked and streaked onto nutrient agar plates (HIMEDIA, M001-100G) and incubated for 16-18 h at 37°C.

2.2.3. Identification of *Vibrio* species

The suspected isolates were further identified According to the protocol provided by ISO/ TS 21872-1 (2007) and ISO/ TS 21872-2 (2007).

2.2.4. Microscopic examination

Films were obtained from pure cultures of the isolated organism. Each film was stained with the Gram staining technique and examined under the microscope to confirm the existence of the organism's special features. *Vibrio* cells appeared short, rigid, Gram–ve, with single flagellum and have comma shape (ISO-TS-21872-1, 2007; ISO-TS-21872-2, 2007).

2.2.5. Biochemical examination

Biochemical identification of the suspected Vibrio isolates (n= 29) was carried out as previously described (ISO-TS-21872-1, 2007; ISO-TS-21872-2, 2007). The following biochemical tests were conducted (Oxidase test, H_2S production test, Detection of L- lysine decarboxylase, Detection of Arginine decarboxylase, Detection of β- galactosidase, Detection of Ornithine decarboxylase, Indole test and Halotolerance test.

2.2.6. Antimicrobial susceptibility testing

The identified *Vibrio* spp. isolates (n=11) were subjected to antibiotic susceptibility testing against 15 of the commonly used antibiotics for treatment of *Vibrio* infection in fish and humans. The antibiotic susceptibility profile of each isolate was performed according to the National Committee for Clinical Laboratory Standards recommendations (NCCLS) by the standard Kirby-Bauer disk diffusion method (CLSI, 2019).

The panel of antimicrobial agents encompassed Ampicillin (AMP, 10µg), Amoxicillin / clavulanic acid (AMC, 30 µg), Cephalothin (CEP, 30µg), Cefoxitin (CX, 30 µg), Cefoperazone 75 (CPZ-75, 75µg), Kanamycin (K, 30µg), Gentamycin (GEN, 10µg), Streptomycin (s, 10µg), Ciprofloxacin (CIP, 5 µg), Tetracycline (TE, 30µg), Doxycycline (DO, 30 µg), Meropenem (MRP, 10 µg), Imipenem (IPM, 10 µg),Nalidixic acid (NA, 30 µg) and Chloramphenicol (C, 30 µg). Incubation of the plates occurred over a period of 16–24 hours at 37°C.Subsequently, we measured the zones of growth inhibition surrounding each antibiotic disc, recording the

values to the nearest millimeter. These zone diameters served as indicators of the isolate's susceptibility and the drug's diffusion rate within the agar medium.

III.RESULTS

3.1. Isolation and identification of *Vibrio* isolates

3.1.1. Colonial and biochemical appearance on Thiosulphate Citrate bile-salt Sucrose agar (TCBS).

As shown in Figure 1, typical colonies of V. mimicus, V. vulnificus and V. parahaemolyticus were appeared as smooth and green (sucrose negative), while colonies of V. cholerae, V. fluvialis and V. alginolyticus were appeared as smooth and yellow (sucrose negative) (Ibrahim et al., 2018). Positive indol test, negative Sucrose except V. cholera, and optimal growth at 3 % NaCl.

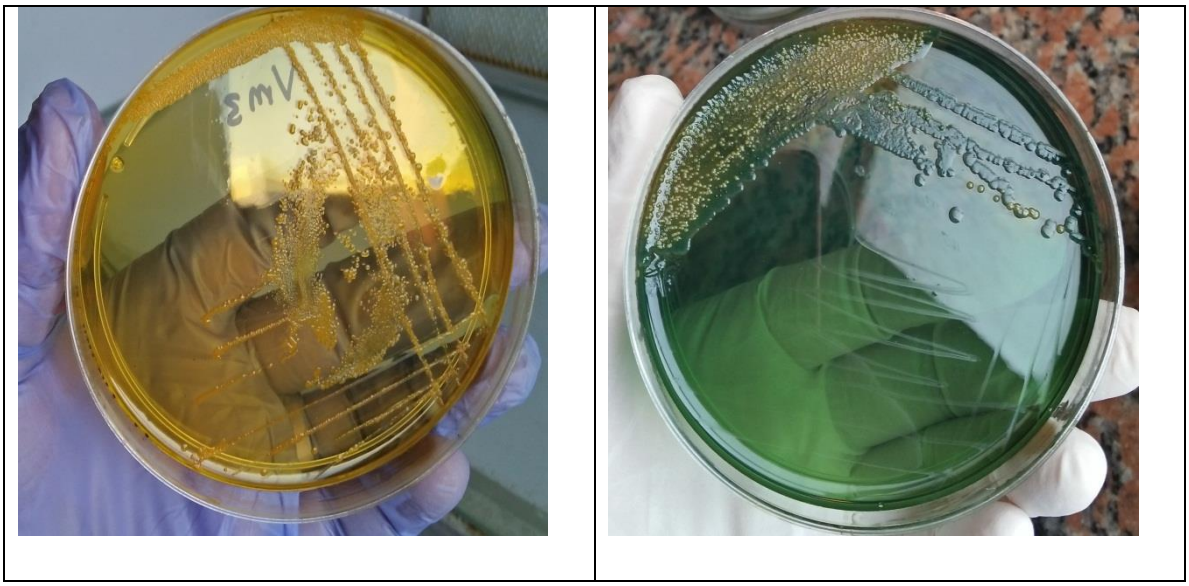

Figure 1. Cultural characters of *Vibrio* isolates on Thiosulphate Citrate bile-salt Sucrose agar (TCBS), (A) for *V. cholera* and (B) for *V. parahaemolyticus*.

3.1.2. Prevalence and serotyping data

The prevalence rate was 55% (11) for *V. mimicus*, 25% (5) for *V. vulnificus*, 20 % (4) for *V. parahaemolyticus*, 20 % (4) for *V. fluvialis*, 10% (2) for V. alginolyticus, 10% (2) for *V. cholera* and 5 % (1) for *V. hollisae* **(Table 1)**. Different *Vibrio* species were isolated with a total prevalence rate of 100% (20/20) with predominance for *the V. mimicus* serotype as illustrated in **Figure 2**.

Table (1): Incidence of *Vibrio* spp. isolated from beef frozen meat.

Isolates	No	%
V. fluvialis	4	(20 %)
V. parahaemolyticus	*4*	(20 %)
V. alginolyticus	*2*	(10 %)
V. cholerae	*2*	(10 %)
V. mimicus	*11*	(55 %)
V.hollisae	*1*	(5 %)
V. vulnificus	*5*	(25 %)

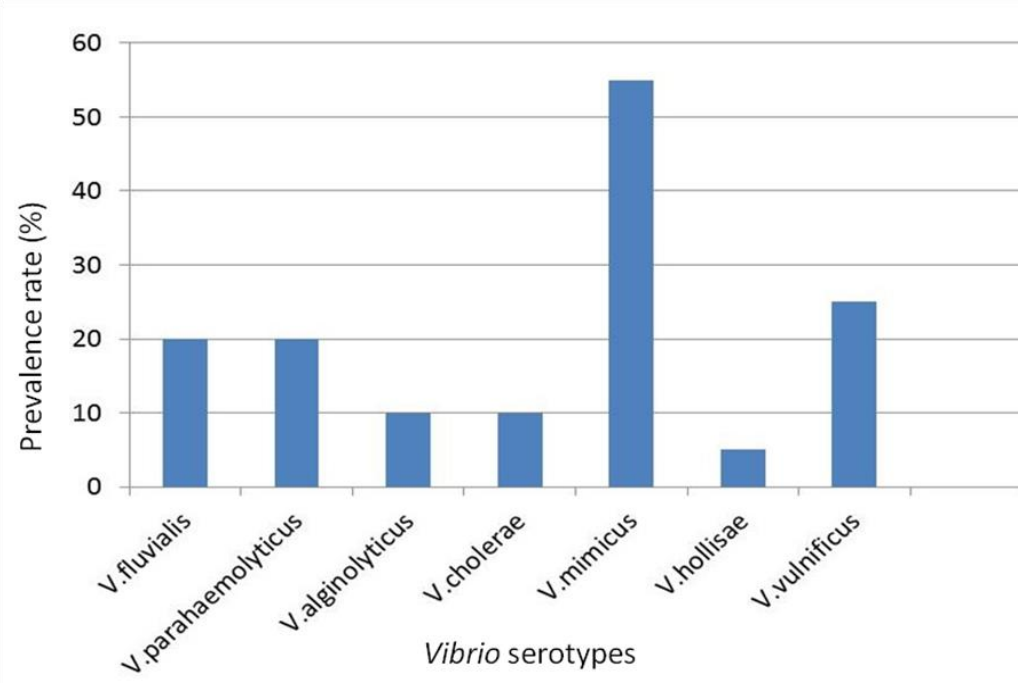

Figure 2. Prevalence rate of different *Vibrio* serotypes in examined beef frozen meat samples.

3.1.3. Antimicrobial Susceptibility pattern

Data of Figure 3 show that most isolates were resistant to Amoxicillin / clavulanic acid (AMC) (90.9%) followed by an Ampicillin (AM)and Cefoxitin (FOX)with percentages of 72.7 and 27.3 %respectively. Moreover, Ciprofloxacin (CIP), Tetracycline (TE), Doxycycline (DO), Meropenem (MEM), Imipenem (IPM) and Imipenem (IPM) represented absolute susceptibility to all isolates and was regarded as a drug of choice for vibrosis infection.

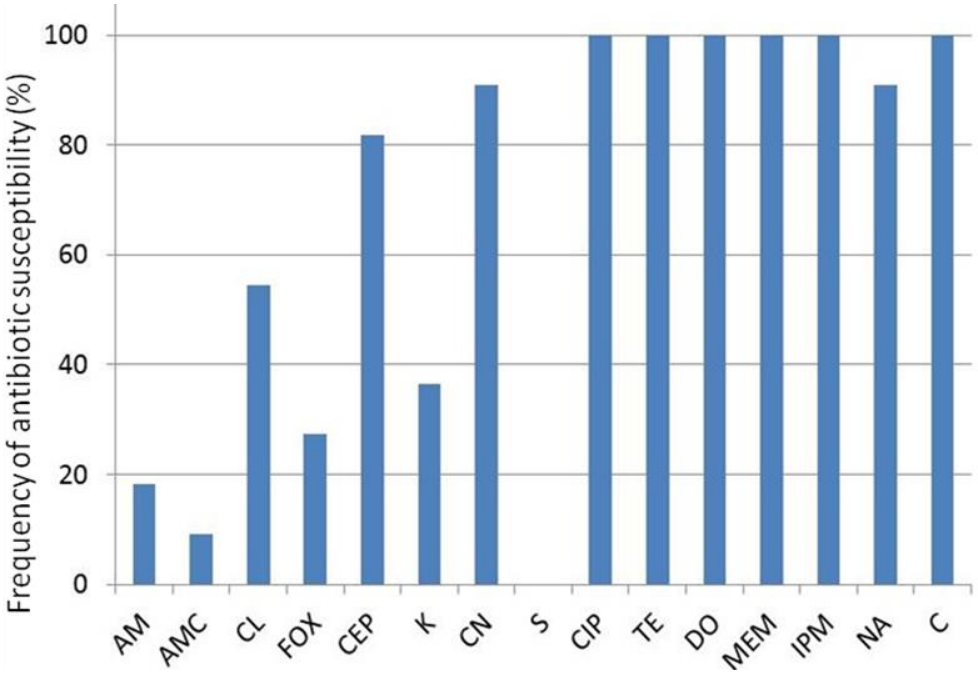

Figure 3. Frequency of antimicrobial susceptibility of *Vibrio* isolates from raw beef frozen meat products. AM: Ampicillin, AMC: Amoxicillin/clavulanic acid, CL: Cephalothin, FOX: Cefoxitin, CEP: Cefoperazone 75, K: Kanamycin, CN: Gentamycin, S: Streptomycin, CIP: Ciprofloxacin, TE: tetracycline, DO: Doxycycline, MEM: Meropenem, IPM: Imipenem, NA: Nalidixic acid, and C: Chloramphenicol.

IV. DISCUSSION

In contrast to fresh packing, which is done at a temperature of -38 to -42°C, chilling is done at a temperature of 0–4°C, and frozen meat in the slaughterhouse area becomes contaminated during the processing steps of deboning, chilling, and freezing. As a result, frozen meat's food quality could be damaged and there would be a high risk of contamination and invasion by potential pathogens **(Azwai *et al.*, 2016).** Accordingly, it is now known that the primary way that cholera spreads to meat is through abattoir water. As a result, in order to break the cycle of transmission, the abattoir should install an efficient water treatment system for public health purposes in order to prevent meat contamination. Additionally, the water should be released into the environment with an appropriate effluent treatment system.

In this study, the prevalence rate of 100 % (20/ 20) in the studied food items. This can potentially affect the quality and safety of the processed meat and present a potential risk to the consumer. The recovery rate of *Vibrio* from meat varied among several countries. In the range of 60.9 % in India **(Sen and Garode, 2018)**, 44.6% were fromlibyia **(Azwai *et al.*, 2016)**. Therefore, this is considered a risk indicator for the increase in pathogenic bacteria, and care must be taken to limit its spread.

Regarding, the antimicrobial susceptibility profile, the isolates were resistant to Amoxicillin / clavulanic acid (AMC) 90.9%, followed by Ampicillin (AM) and Cefoxitin (FOX) with percentages of 72.7%and 63.6 %, respectively. Ciprofloxacin (CIP), Tetracycline (TE), Doxycycline (DO), Meropenem (MEM), Imipenem (IPM) and Imipenem (IPM) represented (100%) more sensitivity to all isolates. In another study, most isolates were susceptible to Gentamycin and Nalidixic acid and absolute resistance was obtained among the isolates against Ampicillin (100%) followed by Ciprofloxacin (91.7%), Kanamycin (72.2%), Chloramphenicol (61.1%) and gentamicin (50 %), **(Ahmed *et al.*, 2018).**

Conclusion:

Based on biochemical and microscopic examination, we conclude that *V.mimicus* is the most prevalent *Vibrio* spp found in frozen meat samples.

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