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Prevalence and Bacteriological Study of Gram-negative bacteria especially *Citrobacter spp.* in Poultry meat from Maeen Area- Sana'a, Yemen

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ABSTRACT

To ascertain the frequency of pathogenic bacteria in Yemeni poultry meat Maeen Area-Sana'a. This study aimed to determine the general microbiological quality characteristics and presence of *Citrobacter* spp. in chicken meat samples obtained from butcher shops. One hundred Fifty (150) chicken meat samples were collected randomly from local markets in Sana'a city from January to April. 2022. Based on using differential and selective media, bacteria were isolated from the samples and characterized by using biochemical tests. Vitek 2 compact was used to confirm the diagnosis. The results of this study showed that, out of 150 poultry meat samples, 140 samples (93%) were positive and 10 samples (7 %) were negatively isolated. Among 302 isolates: *Citrobacter* spp 106 (35.1 %), *Klebsilla* spp 55 (18.2 %), *Proteus vulgaris* 42 (13.9 %), *Sigella* spp 36 (11.9 %), *E. coli* 27 (8.9 %), *Proteus mirabilis* 24 (8%) and *Salmonella* spp 12 (4 %). The study showed that the raw chicken meat samples of Sana'a city, Yemen, were contaminated with Gram-negative potential pathogenic bacteria. We must use new methods of testing and characterization for bacteria. PCR-based methods for identifying pathogens provide more advantageous options for this purpose than conventional testing.

Keywords: *Citrobacter* spp., Gram-negative bacteria, poultry meat, Yemen.

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INTRODUCTION

Foodborne infections are on the rise internationally and particularly widespread among those who consume meals outside the home. They are the main public health and financial burden due to the uncontrolled hygienic preparation of various kinds of food. The WHO defines a foodborne disease as any infective or hazardous condition due to eating contaminated food. (**le Loir *et al.*, 2003).**

A few examples of the foodborne pathogens of animal origin that can contaminate food and spread: *Salmonella* spp., Campylobacter, *E. coli*, *Staphylococcus* spp., *Clostridium*, *Yersinia*, *Listeria*, *Arcobacter*, *Mycobacterium*, *Trichinella*, and *Sarcocystis*. Beef and chicken meat contaminated with fecal organisms may consider essential food hygiene problems, particularly Enterobacteriaceae including *Salmonella* spp, *E. coli*, *Proteus* and *Klebsiella* spp (**Paterson 2006).**

One of the most significant zoonotic bacterial foodborne illnesses worldwide is salmonellosis. Infants, the elderly, and people with compromised immune systems are all susceptible to serious diseases from *Salmonella*. *Salmonella* infections are typically linked to animal excrement or dietary items made from animals. When compared to other food products, chicken, other poultry meat, and eggs are the leading cause of *Salmonella*. (**Vose *et al.*, 2011).**

The family *Enterobacteriaceae*, phylum Proteobacteria, contains the Gram-negative coliform bacteria known as *Citrobacter* species. There are 14 species in the *Citrobacter* genus. However, *C. freundii* and *C. koseri* are the most common ones to infect humans. Numerous *Citrobacter* species have been identified in a variety of habitats, including soil and water, as well as in the animal gut microbiota, including the human intestine. **(John and Bennett, 2020**).

The availability of data on the frequency of Gram-negative bacteria in chicken meat in Yemen is inadequate. So, this study aimed to determine how common Gram-negative bacteria were in chicken meat and whether *Citrobacter* spp was present in various chicken meat samples.

MATERIALS AND METHODS

Samples collection

The present study was done between January and April of 2022. One hundred fifty samples of chicken meat were collected in sterile containers. The samples were collected from various district supply shops in Sana'a ' Maeen neighborhood. The research was carried out at Sana'a Central Health Laboratory's Food Microbiology lab.

Isolation

One gram of each chicken meat sample was dissolved in nine milliliters of distilled water and left for 1 minute. Then, 0.1 milliliters of each sample suspension were inoculated on the *Salmonella shigella* (S.S.) agar medium, which was then incubated at 37 °C for 24 to 48 h. Later, an additional investigation was conducted on the developed colonies.

Identification

The conventional morphological and biochemical assays were used to identify the *Citrobacter* isolates to the species level (**MacFaddin, 2000**). By using the vitek2 compact system, the isolates' identification was verified.

S.S. agar and Xylose lysine deoxycholate (XLD) agar

After being kept for 37°C overnight, the organisms were grown on an S.S. agar medium after 18 h. incubation, *Citrobacter* colonies with black centers appear as yellow colonies on XLD agar. (**MacFaddin, 2000**).

Identification of bacteria by Vitek 2 compact system

The biochemical profile of test isolates was determined with the VITEK system- bioMérieux following the manufacturer's instructions. Incubation for 37°C overnight, colonies showing different morphologies were picked up from each selective plate and tested separately with VITEK for identification.

RESULTS

Bacterial Isolation and Identification

Bacteriological examination of the samples showed Gram-negative bacteria. In one hundred fifty poultry meat samples, 93% (140 samples) were positive and 7 % (10 samples) were negatively isolated (Fig.1).

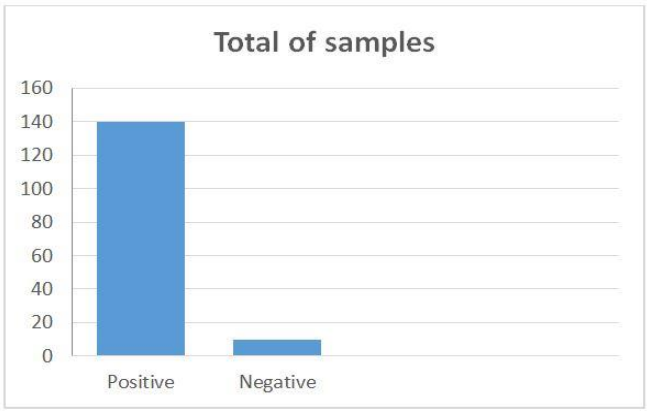

Fig. 1: Total of samples positive and negative bacterial isolates.

The bacteriological culturing expressed colonies' different shapes, colors and consistency using selective media such as XLD and S.S. agar (Tableb1),(Fig. 2-A, B, C). Gram stains showed of these culturing were all Gram-negative bacteria. (Fig. 3).

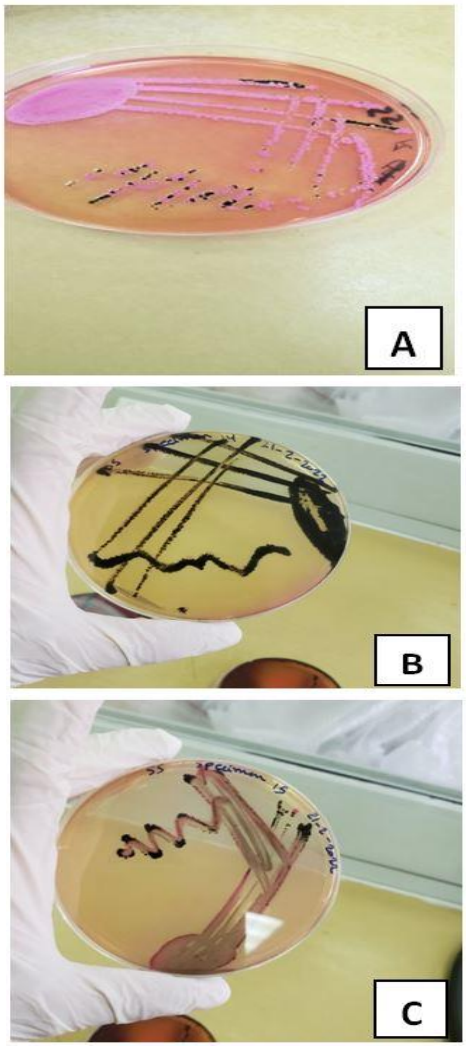

Fig.2 (A, B,C): *Salmonella* spp. on S.S agar at 37°C for 24 hr.

Table 1: Cultural characteristics of selective and differential media:

Organisms	S.S. agar	XLD agar
Shigella	Clear, colorless, transparent	Red colonies
Escherichia coli	Small, pink to red	Large, flat, yellow colonies
Enterobacter, Klebsiella	Larger than E.coli, mucoid, pale, opaque cream to pink	Yellow colonies / Yellow and mucoid colonies
Salmonella	Colorless, transparent, with a black center if H_2S is produced	Red colonies with black centers Red colonies with negative H_2S
Citrobacter, Proteus	Colorless, with a black center	Red to Yellow colonies

Among 302 isolates, it was: a *Citrobacter* spp 106 (35.1 %), *Klebsilla* spp 55 (18.2 %), *Proteus vulgaris* 42 (13.9 %), *Sigella* spp 36 (11.9 %), *E. coli* 27 (8.9 %), *Proteus mirabilis* 24 (7.9%) and *Salmonella* spp 12 (4 %), (Table -2).

Table 2. Bacterial species were isolated from 140 samples:

Total No. of isolate	*Citrobacter* spp	*Klebsilla* spp	*Proteus Vulgaris*	*Shigella* spp	*E. coli*	*Proteus mirabilis*	*Salmonella* spp
302	106 (35.1 %)	55 (18.2 %)	42 (13.9 %)	36 (11.9 %)	27 (8.9 %)	24 (7.9 %)	12 (4 %)

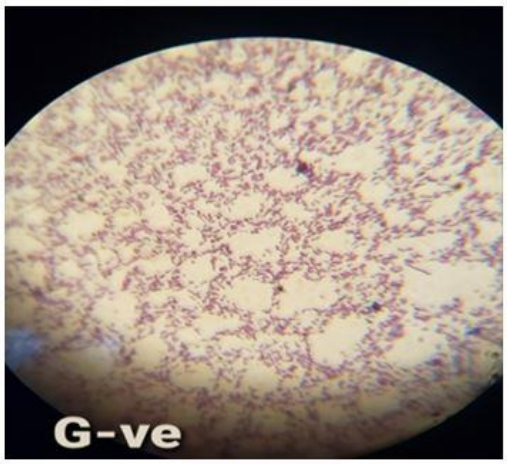

Fig. 3: Gram stain of *Citrobacter* spp.

DISCUSSION

This study observed that *Citrobacter* spp 106 (35.1 %), *Klebsiella* spp 55 (18.2 %), Proteus Vulgaris 42 (13.9 %), *Shigella* spp 36 (11.9 %), *E. coli* 27 (8.9 %), *Proteus mirabilis* 24 (7,9 %) and *Salmonella* spp 12 (4 %), were the major Gram-negative bacteria among 302 bacterial isolates (Table -2 .

Most of these isolates are pathogenic, implying that chicken meat is a common source of foodborne infection. In several other studies conducted in North East India, China, South Korea, Vietnam, and Spain, there is a similar type of Gram-negative bacteria **(Saikia and Joshi, 2010).** *Citrobacter* species were commonly discovered in soil, food, water, animal, and human intestines. Most human cases of *Citrobacter* infection are caused by *C. freundii* and *C. koseri* **(Pepperell *et al., 2002*).**

In this study, the predominance of *Citrobacter* spp. was found to be 35.1%; these magnitudes are comparable with the study conducted by **(Kanamori, *et al.*, 2011**), which reported that 19.3% of *Citrobacter* spp. is a low virulence bacterium and thus can persist in host population for long periods**. (Kanamori, *et al.*, 2011**). They accumulate resistance determinants over time and may evolve into more virulent organisms **(Pepperell *et al., 2002*).**

Klebsiella spp had occurrence as isolates 55 (18.2 %), which is significantly less compared to the result shown in Oklahoma, which is 30%, but close to (**Al-Mutairi, 2011**) (10.7%).

Klebsiella spp. is a pathogen that colonizes humans and animals and is a common pollutant of retail meat. (**Bersisa *et al.*, 2019).** The percentage of

Proteus mirabilis and *Proteus Vulgaris* isolates was more similar to the study by **(Shrestha *et al.*, 2017)** but lower than the study by **(Al-Mutairi, 2011*)*** from Saudi Arabia.

Proteus spp. is considered an indicator of meat contamination during any processing, handling, and storage stages. Suppose the optimal condition for the isolated *Proteus* existed. In that case, typical cases of food poisoning, urinary infection, and other *Proteus*-related human illnesses could happen due to the rapid proliferation of the pathogen **(Al-Mutairi, 2011)**. The increase in food contamination factors and gastrointestinal diseases is associated with an increased risk of non-specific salmonellosis (**Crum– Cianflone, 2008**).

In Yemen and most developing countries, the lack of epidemiology studies of salmonellosis cases is an obstacle to effectively assessing prevalence. Our data corroborate previous studies revealing that chicken meat is an important food source contaminated by *Salmonella* spp. The difference in prevalence data for *Salmonella* spp. between previous studies and the present study might be due to sanitation conditions, methodological differences used to isolate the bacteria, or transportation and storage conditions (**LI *et al.*, 2013**) .

In this study, *Salmonella* spp. was detected in 12 (4 %) raw chicken meat tested using the conventional. Our results were lower than those reported previously at 66% (150/6442.) by **Siriken *et al.*, (2015**) in Ankara, Turkey. However, **Yildirim *et al.*, (2011**) reported a 34% (68/200) contamination rate of *Salmonella* in meat in Turkey. The contamination rate of *Salmonella* in broilers meat varies among countries (**Alvarez-Fernandez *et al.*, 2012).**

In the present study, the prevalence rate of *Salmonella* in poultry meat was lower than the 60% reported in Portugal (**Antunes *et al.*, 2003**) and 67.5% in Thailand (**Lertworapreecha *et al.*, 2012**), and the contamination rates of 55% in Spain and 52.2% in China (**Yang *et al.*, 2011).**

In contrast, the incidence rates of *Salmonella* in poultry meat in South Korea and Pakistan were 3.7% (**Ranhee *et al.*, 2014**) and 5.26% (**Akbar and Anal, 2013**), respectively. These results indicate that poultry meat is an important source of *Salmonella* spp. infections.

CONCLUSION

The obtained results in the present study concluded that the examined chicken samples showed higher significance, where the Gram-negative bacteria among 302 bacterial isolates counted are: *Citrobacter* spp (35.1 %), *Klebsiella* spp (18.2 %), *Proteus vulgaris* (14 %), *Shigella* spp (12 %), *E. coli* (9 %), *Proteus mirabilis* (8%). At the same time, *Salmonella* species were isolated at 4%. The result demonstrates that the unhygienic and poor sanitary conditions under which the meat is being handled are not acceptable from a sanitary point of view. It has further evidence that the undesirable level of contamination might have been acquired from the environment to obtain wholesome, safe, and sound meat.

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