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Research Article

Isolation and Identification of *Helicobacter Pylori* from Poultry Meat in Lahore, Pakistan

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ABSTRACT *Helicobacter pylori* is a gram-negative bacterium that is recognized as a causative agent of major diseases in the gastrointestinal tract, peptic ulcer disease, and gastric cancer. The poultry meat origin has also been a cause of concern due to its zoonotic capability. This study sought to investigate the presence of *H. pylori* in poultry meat tissue samples obtained from Lahore, Pakistan, and further examined the resistance of isolated bacteria to antibiotics. A total of 50 samples were collected from the home-affairs including casual yet expandable retailing facilities and coded samples were cultured on Brucella blood agar under microaerophilic conditions. Biochemical tests including catalase, urease and indole tests helped to detect only 1 sample of *H. pylori* confirming its occurrence and lower prevalence level. The isolate was found to be susceptible to ampicillin, kanamycin, but not streptomycin. 34% of the samples exhibited poly – microbial growth; this might have made it difficult to isolate *H. pylori* in other instances. This suggests that the direct zoonotic impact of poultry meat is low although the potential transmission will persist particularly in areas with poor hygiene. Further research should be considered the geographical expansion of the relationship between *H. pylori* prevalence and the level of antibiotic resistance for better evaluation of poultry meat public health risk.

KEYWORDS Helicobacter, Poultry, Meat, Microbial Resistance, Prevalence

Introduction

Helicobacter pylori is an outstanding pathogen that deserves global attention in peptic ulcer, gastric cancer, and chronic gastritis (Addissouky *et al*, 2023). More than half of the world's population is infected by this gram-negative bacillus which colonizes the gastric mucosa with the prevalence more in the third world countries (Kusters *et al*, 2006). Clinical studies had earlier linked the prevalence of *H. pylori* to bad eating or lifestyle habits (Habbash *et al*, 2022). However, studies have shown that this organism is the causative agent of the condition hitherto known to be ascribed to poor lifestyle choices (Lippi *et al*, 2016). Concern had been raised about the zoonotic spread of the organism, especially through poultry. *H. pylori* and other pathogenic bacteria may find a lucrative and easy unsuspecting reservoir in poultry which is a commonly consumed protective protein.

Poultry meat is protein often eaten by majority with particular attention paid in third world countries where hygiene standards during the slaughter of fowl are mostly ignored (Cartoni Mancinelli *et al*, 2022). Such poor sanitary conditions and subsequent cross contamination, both in the slaughter and the process stage, heightens the chances of spreading bacterial infection (Rouger *et al*, 2017). Studies have already showed *H. pylori* presence in poultry which suggests example of zoonotic dissemination (Saeidi and Sheikhshahrokh, 2016). The economic survey of 2011 and 2012 shows 1% increase in poultry consumption at 25.8 % of total meat produced (Sohaib and Jamil, 2017). This tells the poultry meat consumption is increasing in Lahore which is replacing the red meat day by day. This fuels debate about the concern of poultry meat for food borne pathologies including *H. pylori* related diseases. The situation worsens looking especially at the growing trend of poultry consumption in the urban areas like Lahore.

Though *H. pylori* is a well acknowledged disease globally, there is little information regarding its prevalence in poultry meat in Pakistan. This assists in controlling zoonotic transmission of *H. pylori* which spreads through food sources. The present study is objective of isolation and identification of *H. pylori* from poultry meat in the markets of Lahore. The research also studies the antibiotic

susceptibility of the strains obtained. These findings will serve as an important step towards understanding the risk of poultry meat and the risks that require measures in food safety.

Materials and Methods

Sampling and Study Area

The study was conducted in Lahore, the capital city of Punjab province, Pakistan. Samples were collected from retail poultry stalls points located in 9 towns (Ravi town, Shalimar town, Wagah town, Aziz Bhatti town, Data gunj bakhsh town, Gulberg, Saman-abad, Iqbal town and Nishtar town) and 1 cantonment of Lahore. The samples of different organs such as liver, intestines, heart were collected in separate sterilized polythene bags and placed in ice box and transported at 4ºC to the Biotechnology laboratory, Kinnaird College for Women, Lahore for further diagnostic purposes.

Isolation And Identification

Brucella agar base supplemented with 7% sheep blood was used for the isolation of *H. pylori* from poultry meat. Sterile cotton swab was used for the surface streaking of the collected samples. After inoculation of samples, Petri plates were then tightly packed with parafilm and placed in a desiccator with burning tea candles in it for creating microaerophilic conditions. The desiccator was then placed in incubator at 37 ºC for 5 to 7 days.

Microscopic Identification

Colonies having morphology having typical small circular colonies with 0.5 to 2mm in diameter appeared after 5 to 7 days of incubation were identified as isolates. Gram staining was performed as initial step in bacterial culture and identification. Curved rods or spiral shaped, pink-coloured bacteria were considered as potentially positive for *Helicobacter* genus.

Biochemical analysis

These colonies were then picked for gram-staining and different biochemical tests (such as catalase, urease, and indole test). In catalase test, bacterial culture was mixed with catalase enzyme on a clean glass slide. In urease test, urea agar base slants were prepared by adding 2.4 grams of urea agar base in 95 ml of distilled water followed by addition of urea solution. Upon solidification, these slants were inoculated with bacterial culture and incubated at 37°C for 24 hours. Positive results were indicated by change in colour to bright pink. For indole test, Tryptone broth was prepared by adding 1 g tryptone and 0.5 grams of NaCl in 100 ml of distilled water. Tryptone broth was inoculated with bacterial culture and incubated for 24 hours at 37°C followed by addition of Kovac's reagent. A pink ring formation was indicator of positive result.

Antimicrobial resistance

Antibiotic resistance was determined by pouring agar plate method using antibiotics such as ampicillin, kanamycin, and streptomycin. Concentrations of 100 mg/ml, 60 mg/ml, and 50 mg/ml of antibiotics were used for ampicillin, kanamycin, and streptomycin, respectively. After preparing the plates, the positive culture was evenly spread on the media plates using glass spreader and incubated for 24 hours at 37°C.

Results

A total of 50 samples from poultry retail stalls were collected from each town of Lahore district. These samples consisted of liver, heart, and intestines from one bird of shop. Only one sample was found positive based on culturing technique. This sample showed characteristic typical round colonies of *H. pylori* (Fig. 1). Remaining 49 samples showed no cultural growth on the plates.

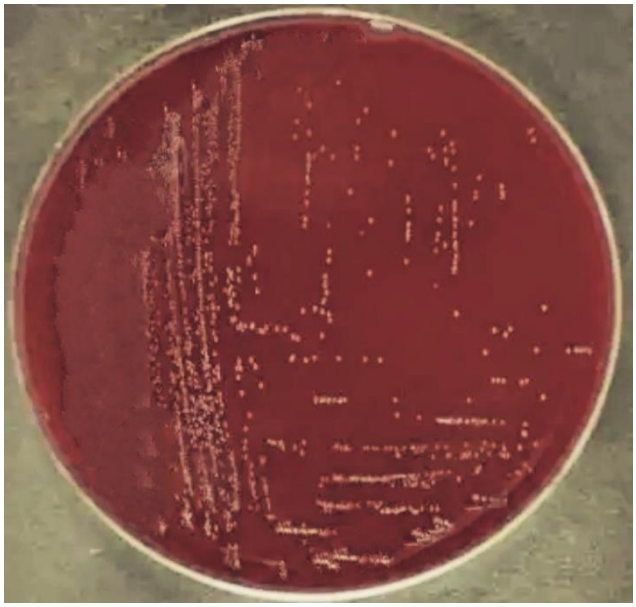

Fig. 1: Typical small circular colonies with 0.5 to 2mm in diameter appeared after 5 to 7 days of incubation were identified as isolates. On the other hand, no clear growth was observed in other plates.

Among all the towns sampled, only Shalamar town was identified with the positive sample (Fig. 2). All the organs sampled from this bird showed the presence of *H. pylori.* Remaining 49 sampled birds from all the towns were found negative.

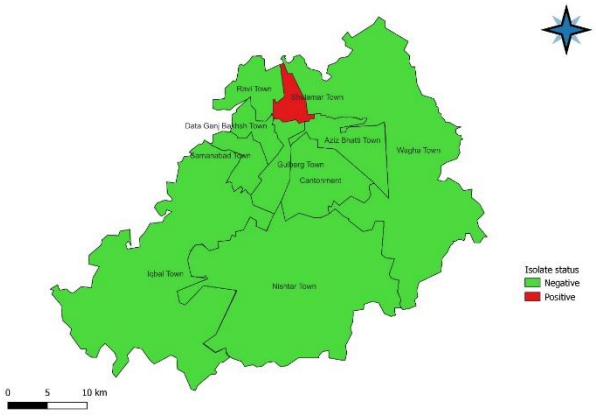

Fig. 2: Map of Lahore district depicting the positive percentage of isolates from poultry samples. Lahore district has 9 towns and 1 cantonment area.

Gram-staining and Biochemical tests

To further identify the bacterial characteristics, gram-staining and biochemical tests such as catalase test, urease test, and indole test were performed (Fig 3). Curved rods or spiral shaped, pink-coloured bacteria were observed under microscope which conforms the presence of *H. pylori.* The presence of bubbles meant the organism tested positive for catalase enzyme when a few drops of hydrogen per oxide were added to the culture slides. The formation of pink ring on addition of Kovac's reagent in tryptone broth inoculated with positive culture confirmed the presence of gram-negative *H. pylori.* The most definitive test for the conformation of *H. pylori*, the colour changes from yellow to bright pink shows the presence of *H. pylori.*

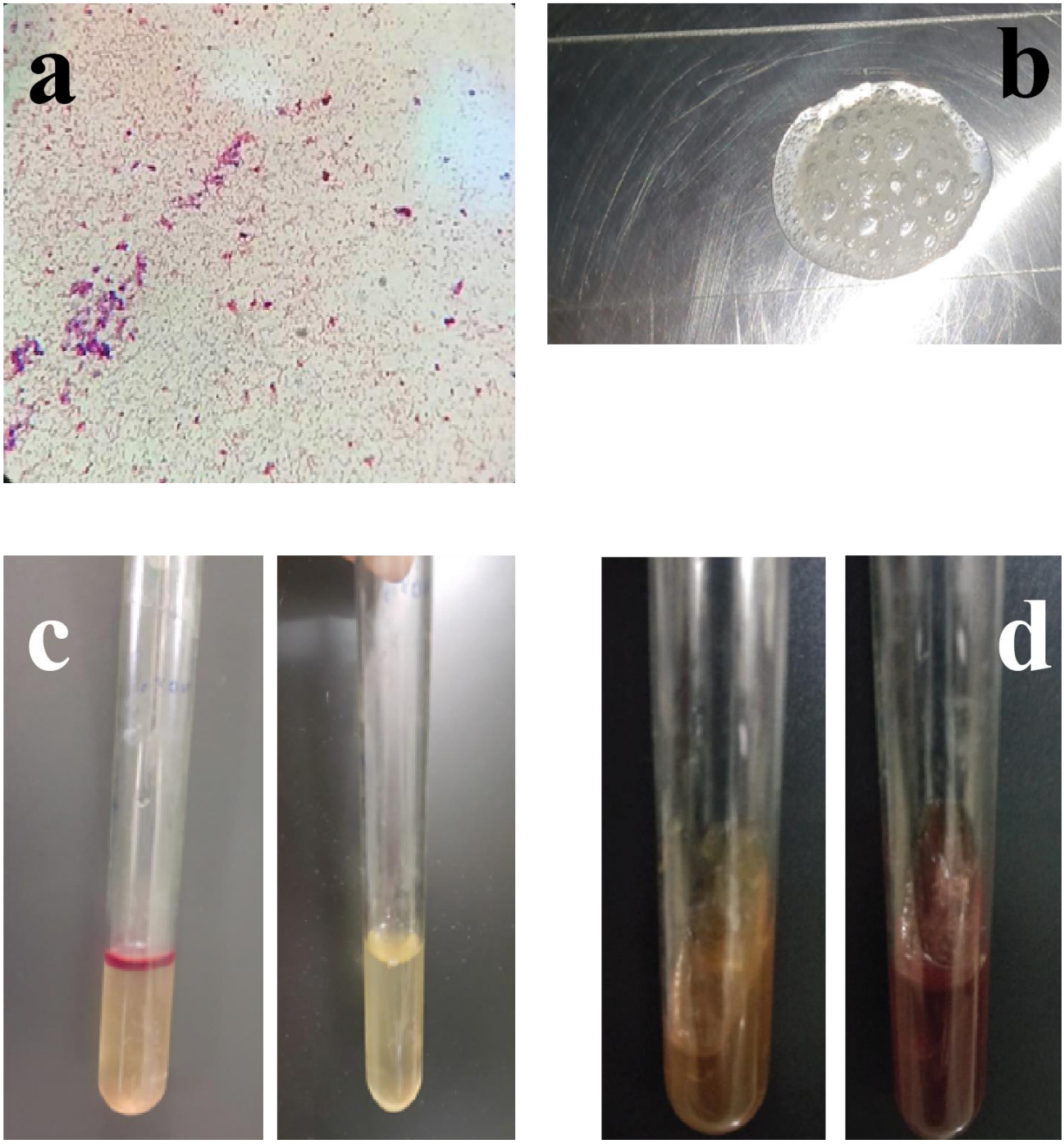

Fig. 3: Biochemical analyses of the bacterial culture (a) Curved rods or spiral shaped, pink-coloured bacteria were observed under microscope which conforms the presence of H. pylori **(b)** Catalase test to detect presence of gram-negative H. pylori. Oxygen bubbles formation showed the presence of bacterium. **(c)** Indole test to detect the presence of gram-negative H. pylori. The first figure on the left shows positive result and the second figure on the right is for negative result. **(d)** Urease test to conform the presence of H. pylori. The first figure on the left is showing negative result while second figure on the right is showing positive result.

Antibiotic Testing

Antibiotic resistance was determined using ampicillin, kanamycin, and streptomycin antibiotics with the concentrations of 100 mg/ml, 60 mg/ml, and 50 mg/ml, respectively. These concentrations were prepared in sterilized water and injected in media at semi-solid stage followed by thorough mixing. These plates were then incubated for the growth. No growth was observed in all the plates containing these antibiotics at these mentioned specific concentrations. This result showed that the isolated sample of *H. pylori* is sensitive to ampicillin, kanamycin, and streptomycin antibiotics at the concentrations of 100 mg/ml, 60 mg/ml, and 50 mg/ml, respectively.

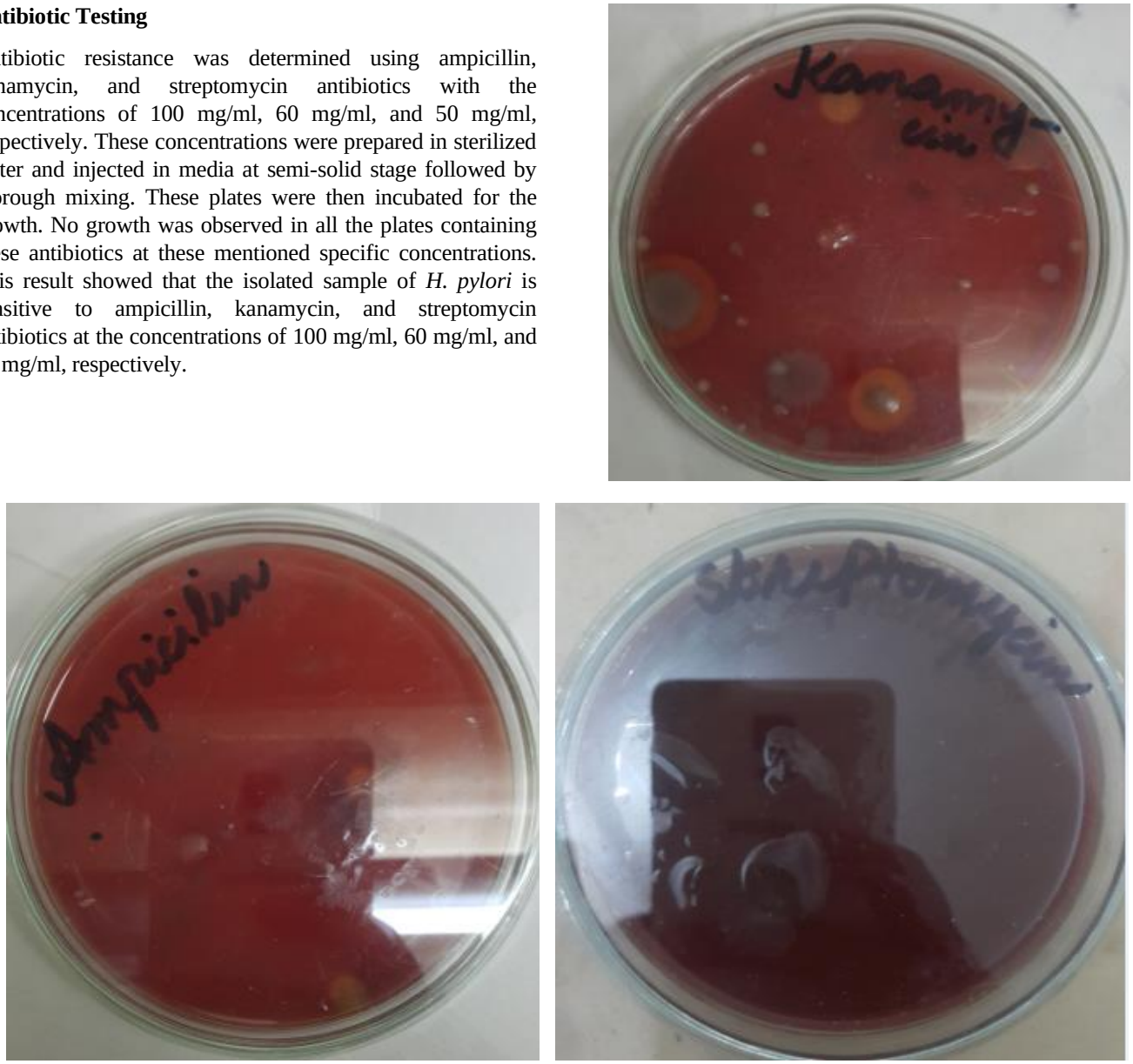

Fig. 4: *H. pylori* sensitivity towards ampicillin, Kanamycin, and streptomycin.

Discussion

The isolation of *H. Pylori* from meat in this study raises alarm on how such bacterium may be transferred due to animal to human contact. Of the *H. pylori* samples that were cultured from the tissues and biological fluids only one sample was positive whereas other studies have reported higher prevalence rates indicating that *H. pylori* colonizing poultry is a big problem as a reservoir (Elrais *et al*, 2022). The low prevalence in this study may be since *H. pylori* is not easy to isolate because it has a very complicated media for its growth (Kusters *et al*, 2006). Further, it has also been observed that such risks have also been caused due to contamination during slaughter and processing. Hence, the risk of zoonosis can be lowered by improving hygiene conditions in poultry production.

H. pylori often come at low isolation in this research which also maybe be due to the use of conventional culturing method (Malfertheiner *et al*, 2023). Enhanced molecular techniques like that of PCR may help improve the *H. pylori* detection rate in poultry products. In addition, others factors such as sample treatment, surrounding environment, and the turnover of organisms probably could have contributed to the failures to isolate bacteria (Addissouky *et al*, 2023; Ali and AlHussaini, 2024). The results of this study agree with the findings of other studies which have reported heterogeneous isolation rates of *H. pylori* from poultry. If a better prevalence of *H. pylori* in poultry products is envisaged, a further investigation on this more thorough, focusing on larger population sizes and better detection limits should be performed.

Antimicrobial resistance is an increasing threat concerning *H. pylori* eradication therapies (Smith and Yamaoka, 2023). In this research, the susceptibility of the isolate was high towards the utilized antibiotics. It is suggesting that these classes of antibiotics are still active against those infections. However, other authors have demonstrated that *H. pylori* is able to acquire resistance toward wide spectrum antibiotics that are commonly used especially in areas of antibiotic abuse (Wang *et al*, 2019; Smith and Yamaoka, 2023). Knowing the

patterns of antibiotic resistance in *H. pylori* and their variants is important in making informed decisions on the treatment and containment of resistant variants. This study has few limitations such as excessive contamination led to loss of some samples which shows the need of specialized media. Absence of specialized media led to the polymicrobial growth and hence limits the growth of required bacteria.

In conclusion, *H. pylori* was isolated from poultry meat which represents a risk to human health as it is isolated in case of human intestinal and liver diseases. Further research, with greater and more diverse sample size, can be partaken to estimate the prevalence. *H. pylori* incidence can be better detected using PCR as compared to old culturing techniques.

Declaration of Competing Interest

The authors declare that they have no competing or conflict of interests.

Author Contributions:

Zainab Masood: Conceptualization, Methodology, formal analysis, Writing—original draft preparation. **Tanveer Majeed:** Conceptualization, Methodology, formal analysis, writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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