Saudi Journal of Biological Sciences xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Helicobacter pylori in a poultry slaughterhouse: Prevalence, genotyping and antibiotic resistance pattern

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ARTICLE INFO

Article history:
Received 2 November 2017
Revised 2 January 2018
Accepted 1 February 2018
Available online xxxx

Keywords:
Helicobacter pylori
Virulence genes
Antibiotic resistance
Chickens
Egypt

ABSTRACT

Although *Helicobacter pylori* (*H. pylori*) is a highly significant pathogen, its source remains unclear. Many people consume chicken daily as a source of animal protein worldwide; thus, hygienic methods of supplying chickens for consumption are critical for public health. Therefore, our study examined the distribution of the *glmM* (*ureC*), *babA2*, *vacA* and *cagA* virulence genes in *H. pylori* strains in chicken meat and giblets (gizzards and livers) and the resistance of the strains to various antibiotics. Ninety chicken meat, gizzard and liver samples were obtained from a semi-automatic abattoir in Sadat City, Egypt, and were cultured and preliminarily analyzed using biochemical tests. The presence of the *ureC*, *babA2*, *vacA* and *cagA* genotypes was tested for in samples positive for *H. pylori* by multiplex polymerase chain reaction (Multiplex-PCR). The resistance of *H. pylori* to various antimicrobial drugs was tested using the disc diffusion method. In total, 7 of the 90 chicken samples were positive for *H. pylori* (7.78%); in 3/7 (42.85%) samples, the bacteria were found in the chicken liver, while the bacteria were found in the meat in 2/7 (28.57%) and in the gizzard in 2/7 (28.57%) samples. The total prevalence of both the *ureC* and *babA2* genes in the isolated *H. pylori* strains was 100%, while the prevalence of the *vacA* and *cagA* genes was 57.1% and 42.9%, respectively. The resistance of *H. pylori* to the antibiotics utilized in our study was 100% for streptomycin; 85.7% for amoxicillin and penicillin; 71.4% for oxytetracycline, nalidixic acid and ampicillin; 57.1% for sulfamethoxazole and erythromycin; and 42.9% for neomycin, chloramphenicol and norfloxacin. In conclusion, the chicken meat and giblets were tainted by *H. pylori*, with a higher occurrence of the *ureC*, *babA2*, *vacA* and *cagA* genotypes. Future investigations should investigate the resistance of *H. pylori* to various antimicrobial agents in Egypt.

1. Introduction

Chickens are an important economic source of animal protein for humans (Ammar et al., 2015; Hussain et al., 2015). In restricted slaughtering facilities, chickens are slaughtered, plucked and commonly eviscerated by hand. During evisceration, the carcass is evacuated, the visceral organs are removed, and the liver, heart and gut are collected (Ammar et al., 2015). These organs might be tainted by the spillage of the intestinal contents. After evisceration, the carcasses are washed with water, which may be a primary source of microbial contamination (Arnold, 2007). Millions of people consume chicken daily as a source of animal protein worldwide; thus, hygienic methods of supplying chickens for consumption are extremely relevant to public health.

Helicobacter species are gram-negative, microaerophilic spiral bacterial pathogens that can be exceedingly pathogenic and have been observed to settle in the biliary tract and gut in various animals. According to their favored site of colonization, these

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2018.02.002
1319-562X/

organisms are classified as gastric or enterohepatic Helicobacter (Stanley et al., 1994). These two groups are considered zoonotic microorganisms (Josenhans et al., 2000). In general, during infection by the gastric Helicobacter group, the bacteria colonize the stomach; the enterohepatic Helicobacter group principally colonizes the distal portion of the digestive system and biliary duct (Hassan et al., 2014). *Helicobacter pullorum (H. pullorum)*, which was first isolated from the cecum of apparently healthy domestic fowls and the small intestine and liver of fowls with severe gastroenteritis and hepatitis (Stanley et al., 1994; Zanoni et al., 2007; Qumar et al., 2017), is a member of the enterohepatic Helicobacter group. Furthermore, *Helicobacter hepaticus* (*H. hepaticus*), *Helicobacter canis* (*H. canis*), *Helicobacter bilis* (*H. bilis*) and *Helicobacter cinaedi* (*H. cinaedi*) were isolated from chickens (Taylor et al., 2003; Young et al., 2004).

Helicobacter pylori (*H. pylori*) is a major human pathogenic bacterium associated with stomach cancer and duodenal ulcers (Wong et al., 2004; El Dairouty et al., 2016). Data regarding the prevalence and spread of infection caused by *H. pylori* are essential to control its spread and distinguish high-risk individuals, particularly in districts with an unexpected incidence of gastritis and stomach cancer (Safaei et al., 2011; Rahimi and Kheirabadi, 2012; Momtaz et al., 2014; Mousavi et al., 2014). Although *H. pylori* strains have been previously isolated from various food products, the significant role of foods of animal origin in the spread of *H. pylori* infection remains unclear (El Dairouty et al., 2016; Hemmatinezhad et al., 2016).

The pathogenicity of *H. pylori* is related to virulence factors. Sicinschi et al. (2008) showed that *H. pylori* is genetically mutable, and certain virulence genes are only identified in certain populations. Multiplex polymerase chain reaction (Multiplex-PCR) has been used to identify *H. pylori* isolated from various medical samples. Numerous virulence genes in *H. pylori* strains, such as the urease C (*ureC*), cytotoxin-associated A (*cagA*) and vacuolating cytotoxin (*vacA*) genes, have been identified and may play a role in the development of infection caused by *H. pylori* (Erzin et al., 2006). The *ureC* gene of *H. pylori* encodes a phosphoglucosamine mutase that was recently renamed *glmM*. De Reuse et al. (1997) considered this gene a "housekeeping" gene that contributes to the development and growth of the bacterial cell wall. The *cagA* gene is present in approximately 50% of all isolates of *H. pylori* and is responsible for inflammation in the gastric mucosa, the production of interleukin-8 (IL-8) and the pathogenesis of gastric cancer (van der Ende et al., 1998). In addition, Chomvarin et al. (2008) demonstrated that the *vacA* gene is found in all isolates of *H. pylori* and is responsible for the pathogenesis of stomach carcinoma and ulcers by damaging the gastric mucosa. The *babA2* gene is a membrane protein in *H. pylori* that contributes to the binding activity to the gastric mucosa (Pride et al., 2001). Consequently, the molecular genotyping of *H. pylori* using Multiplex-PCR is considered an intensive method for determining its pathogenicity.

Due to the unexpected resistance of *H. pylori* against several antimicrobial agents, treatment is another significant strategy to prevent the spread of infection in the population (Mégraud, 2004). The resistance of *H. pylori* to various antimicrobial drugs differs by location and appears to be increasing over time in many regions (Meyer et al., 2002; De Francesco et al., 2010; Graham, 2015). Furthermore, using the multiple antibiotic resistance (MAR) index is considered an economic and effective method for bacterial source tracking. This index was previously investigated by Krumperman (1983), who reported that an index of 0.2 indicates a higher incidence of infection where antimicrobial agents are frequently used. To date, no studies investigating the antimicrobial resistance of *H. pylori* isolated from edible and non-edible chicken organs in Egypt have been published. The significance of *H. pylori* and the epidemiology of this pathogenic bacteria in Egypt remain unclear. Animal-derived foods, particularly chicken, should be considered to prevent and control *H. pylori* infection in humans. Therefore, the current study examined *in vitro* the spread of the *glmM*, *babA2*, *vacA* and *cagA* virulence genotypes and their resistance to various antibiotics in *H. pylori* strains isolated from the meat and giblets of broiler chickens.

2. Materials and methods

2.1. Sample origin

Ninety chicken specimens, including meat (n = 30), gizzard (n = 30) and liver (n = 30) samples, were collected from a semi-automatic abattoir in Sadat City, Menoufia Governorate, Egypt, and were examined in this study. Each specimen was placed in a special water-resistant sterilized plastic bag. The specimens were obtained from the meat, livers and gizzards, including the jejunum, cecum and colon, for isolation and molecular identification by Multiplex-PCR. All specimens were kept at –80 °C until further investigation.

2.2. Identification of Helicobacter species

2.2.1. Colony morphology and gram staining

Typical colonies of *Helicobacter* incubated for 5–7 days on *Helicobacter Pylori* Special Peptone (HPSP) agar medium appeared as clear, circular colonies with a diameter of 0.5–2 mm. These colonies were transferred to slants and gram-stained to visualize the gram-negative, S- or C-shaped organisms. Rod and coccoid shapes were observed. The pure colonies were subjected to further identification using biochemical, molecular and antibiotic sensitivity tests. The *H. pylori* ATCC ® 43,504 strain was utilized in the current investigation as a reference strain.

2.2.2. Biochemical analysis of H. Pylori by urease, oxidase and catalase tests

To rapidly identify *H. pylori*, the urease test was performed according to the method previously described by MacFaddin (2000). A pure culture of the tested organism was streaked onto the whole surface of a urea agar plate. The test tubes were incubated at 37 °C in ambient air for 18–24 h. The oxidase test was also performed to biochemically identify *H. pylori*, which produces cytochrome oxidase enzyme. The oxidase activity (blue/purple color) was evaluated in all isolates using oxidase test strips (Sigma-Aldrich, USA). Moreover, the catalase activity in the isolated strains was examined using the drop technique. In brief, hydrogen peroxide (H_2O_2) was added to a pure colony, which was then directly transferred to a slide. The formation of oxygen bubbles was considered a positive result.

2.2.3. Genotypical identification of H. Pylori by Multiplex-PCR

2.2.3.1. Primer sequences. 16S rRNA was applied to detect the Helicobacter species. The *UreC* (*glmM*), *babA2*, *cagA* and *vacA* genes were utilized to molecularly characterize *H. pylori*. All oligonucleotide sequences were designated by Pharmacia Biotech Company (Table 1).

2.2.3.2. DNA extraction. The *H. pylori* DNA was extracted as described by Shah et al. (2009). Briefly, 2 or 3 colonies of overnight culture were inoculated in micro-centrifuge tubes containing 120 µl of phosphate buffer saline (PBS) and mixed carefully for 2 min. All tubes were boiled for 15 min at 100 °C, cooled and centrifuged

Please cite this article in press as: Hamada, M., et al. *Helicobacter pylori* in a poultry slaughterhouse: Prevalence, genotyping and antibiotic resistance patternHelicobacter pylori –>. Saudi Journal of Biological Sciences (2018), https://doi.org/10.1016/j.sjbs.2018.02.002