Review Article

Epidemiology and Pattern of Antibiotic Resistance in *Helicobacter pylori*: Scenario from Saudi Arabia

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ABSTRACT

Helicobacter pylori is recognized as a major cause of gastritis, peptic ulcer, gastric cancer, and mucosa-associated lymphoma. Infection with this gram-negative microaerophile has been treated using combination of antibiotics and proton pump inhibitors for different gastrointestinal diseases. The most commonly used treatment is triple therapy which consists of administration of a proton pump inhibitor, clarithromycin, and amoxicillin. Many factors contribute to treatment failure, but one of the main reasons is development of bacterial antibiotic resistance. The percent prevalence of antibiotic resistance varies among different countries; it appears to be partly determined by the geographic factors and its ability to undergo frequent homologous recombination. The aim of this paper is to review the prevalence of *H. pylori* infection, association of clinical outcomes with *H. pylori* genotypes, and current status of antibiotic resistance in *H. pylori* in Saudi Arabia. It also discusses the different alternative approaches for the treatment of *H. pylori* using antibiotics. In addition, association of antibiotic resistance with *H. pylori* virulent genotypes in Saudi population and its underlying resistance mechanism will also be discussed.

Key Words: Antibiotic resistance, *Helicobacter pylori*, prevalence, Saudi Arabia, virulence genotypes

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Helicobacter pylori is a spiral-shaped, gram-negative opportunistic pathogen that infects more than 50% of the world population.[1] The rate of prevalence observed during the last two decades is highly variable and largely depends on geography, socioeconomic factors, personal hygiene, age, etc. Lifelong infection is considered as a major risk factor for the development of clinically significant disease.[2] It appears that approximately 1% of infected people develop gastric cancer.[3-6] Nonetheless, this suggests that despite the high colonization rate, it causes almost no harm to most of the people who are experiencing infection. The possible subtext for this varied pathogenecity is its tremendous genetic diversity associated with frequent mutation and allelic recombination.[7,8]

The post genomic era of *H. pylori* is quite flourishing wherein the mechanism underlying its genetic diversity, persistent survival, host adaptation, antimicrobial resistance, and varied pathogenesis is recognized. *H. pylori* possesses many strain-specific virulent genes[9,10] apart from the classical virulence factors such as outer membrane protein (OMP), urease (UreA), cytotoxin associated gene A (*cag*A), and vacuolating toxin (*vac*A). Amongst these, *cag*A and *vac*A were considered as stable entities to determine the clinical outcomes.[1,11,12] Eventually, inconsistent observations were reported regarding the association of these genotypes (*cag*A and *vac*A) with the severity of disease from different geographic regions,[13] which suggests that virulence of the genotypes is not a fixed property of the bacterium and that host-specific adaptation operates by selective inactivation of certain virulence genes.[14] Nonetheless, similar mechanism is likely responsible for the differential antibiotic resistance in the bacterium globally.

Given the clinical manifestations, most of the *H. pylori* induced diseases are managed by administration of multidrug regimens,[8] which basically aim to treat peptic ulcer and eventually reduce the risk of developing gastric cancer. This includes triple therapy or quadruple therapy. Triple therapy consists of administration of two antibiotics, such as clarithromycin plus amoxicillin, and a proton

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pump inhibitor (PPI) like omeprazole, lansoprazole, or rabeprazole for a week.[15-17] If resistance to clarithromycin and amoxicillin is more than 20% in the region, sequential therapy or quadruple therapy is advised. In quadruple therapy, a single-triple preparation capsule containing bismuth citrate, metronidazole (MTZ), and tetracycline with PPI is given.[16] While in case of sequential therapy PPI, amoxicillin clarithromycin, and tinidazole are prescribed in a sequential order.[18] Alternatively, use of combination of other antibiotics (multidrug therapy) such as levofloxacin, tetracyclines, fluoroquinolones, and rifamycins was also proposed, but undeniably *H. pylori* resistance to these drugs was also reported by some studies.[15,19]

Several reasons have been proposed to explain the poor efficacy of treatment regimens, inevitably leading to emergence of resistant phenotypes and recurrence of infection,[19] for example, considerable use of antibiotics in clinical practice,[20-22] improper utilization of antimicrobial agents, and the bioavailability of drug in the lumen. In any case, the therapeutic management of *H. pylori* infection still remains an unresolved public health issue worldwide including Saudi Arabia. Hence, on this basis, our aim is to review the prevalence of *H. pylori* resistance to various antibiotics and its underlying resistance mechanism in Saudi Arabia in comparison to the global scenario. This will possibly help clinicians to improve the efficacy of therapeutic regimen locally. Also, the possible association of virulent genotype with antimicrobial resistance, if any, is summarized.

LITERATURE SEARCH

A systematic computer-assisted search was performed concerning the prevalence of *H. pylori* and its resistance toward different antibiotics in Saudi Arabia in comparison to the global scenario using PubMed (http://www.ncbi.nlm.nih.gov/). Additionally, a search was also carried out to analyze the possible association of virulent genotypes with antibiotic resistance. Full-length articles were retrieved for all the relevant studies and the data were extracted, analyzed, and discussed in the review. Data published in the form of abstract were not considered in the review.

PREVALENCE OF *H. PYLORI* INFECTIONS IN SAUDI POPULATION

The epidemiology of *H. pylori* infection demonstrates evident variation between developing countries (80%) and developed countries (25%).[1,23,24] Globally, this variation in prevalence is believed to be socioeconomically driven and depends on the rate of acquisition in the first 5 years of life.[3,25-27]

In the developing countries, acquisition of *H. pylori* in the first 5 years of life is markedly higher as compared to the developed countries, possibly due to good hygiene practices. Observation contrary to this was noted in the developed countries wherein infection generally remains considerably lower in children but slowly rises with increasing age.[11,28] A plethora of studies reporting on the geographic variation in *H. pylori* prevalence have summarized age of bacterial acquisition, period of persistent infection, and rate of eradication as the pivotal factors. Other factors attributed for this unequal geographic burden include age, gender, genetic predisposition, hygiene practices, different ethnicities, etc. Nonetheless, variation also appears between people of different ethnic groups, age, and gender within the same country as well.[25,29,30] [Table 1].

Saudi Arabia is a developing country with varied ethnicity and socioeconomic status. The prevalence of *H. pylori* infections in this region has been widely explored. In 1990, Al Moagel *et al*., reported that 40% of the Saudi population in the age group of 5-10 years and 70% of people >20 years of

Table 1: Global scenario for the percent prevalence of *Helicobacter pylori* infection

Country	Age group (years)	Prevalence of *H. pylori* (%)
India	0-4	22
	10-19	87
	>20	80-88
Bangladesh	0-4	50-60
	8-9	82
	>20	>90
Egypt	0-3	50
	>20	90
Libya	1-9	50
	10-19	84
	>20	94
Turkey	6-17	64
	>20	80
Saudi Arabia	5-9	40
	>20	80
Europe (eastern)	>20	70
(Western)	>20	30-50
Albania	16-64	70.7
Bulgaria	1-17	61.7
Czech republic	5-10	42.1
Estonia	25-50	69
Germany	50-74	48.8
Iceland	25-50	36
Netherlands	2-4	1.2
USA	20	30
Canada	5-18	7.1
	>20	23-30
Ethiopia	2-4	48
	6	80
	>20	95
Nigeria	5-9	82
	>20	70-90

age had *H. pylori* infection, which makes it one of the highest endemic areas in the world.[31] In contrast to this report, Marie observed in 2008 that *H. pylori* seroprevalence was 67% and increases with age, wherein females showed higher prevalence of *H. pylori* infection than males.[32] Prior to this, a study was carried out in healthy individuals of Makkah which reported that *H. pylori* infections are acquired at an early age and reach 61% as the age advances, and so, it recommended a nationwide epidemiological survey.[33]

Concurrent to this study, in 2009, Salih described that in Saudi Arabia, around 32.4% children below the age of 10 years are infected with *H. pylori*.[3] Among the various contradicting reports, a study on the prevalence of *H. pylori* in developing countries by Hunt *et al.* in the year 2010 reveals that in Saudi Arabia, the prevalence of *H. pylori* infections has increased remarkably to 80% in adults and decreased to 40% in children of age between 5 and 9 years.[28] Epidemiological studies on *H. pylori* give contrasting results and are often marked with methodological inconsistencies, albeit it can be concluded that *H. pylori* infections are less among children, but the percentage is high among adults. The population of Saudi Arabia is 27 million including 8.4 million expatriates;[34] the aforementioned studies do not specify whether the study population was homogenous or not.

H. PYLORI GENOTYPES AND THEIR ASSOCIATED CLINICAL OUTCOMES IN SAUDI ARABIA

The major virulence factors of *H. pylori* studied widely include *cag*A product, *vac*A, and induced by contact epithelium (*ice*A).[11] Around 60-80% of *H. pylori* isolates possess *cag*A, which expresses a highly immunogenic protein called cagA. Patients infected with *H. pylori cag*A + strains are known to develop clinically significant diseases such as peptic ulcer or gastric cancer. The presence of *cag*A also strongly correlates with the expression of *vac*A.[11,35,36]

The gene encoding vacA has a mosaic structure consisting of one of three signal sequence types (s1a, s1b, s2) and one of two mid-region types (m1 and m2). The *ice*A gene has two main variant alleles, *iceA1* and *ice*A2. The function of this gene is still being explored. The *cag*A, *vac*A, and *ice*A genotypes and their associated clinical outcomes have been studied in different geographic regions.[37,38] For example, in Jordanian population, *ice*A2 (73.6%) was the predominant genotype and *vacAs2* (54.7%) was more frequently found than *vacAs1*, while *cag*A genotypes were very less (26.4%). Another study in Jordan found the correlation between genotypes and clinical outcomes. Patients with gastritis and atrophy showed infection with *vacAs1* genotype. *vacAs2*, *vacAm2* with erosion, and *cag*A genotypes were significantly correlated with duodenitis and *ice*A2 was correlated with peptic ulcers and gastritis.[39,40] Another study in Basra (Iraq) reported that 45.4% and 45.4% patients with duodenal ulcers and gastritis, respectively, were positive for both *cag*A and *vac*A genes.[4]

Similarly *cag*A, *vacAs1bm1*, and *ice*A2 genotypes are predominant in USA, wherein peptic ulcer patients showed a predominance of *iceA1* alleles.[40] In India, genotype diversity prevails; Chennai (South India) reported predominance of *cag*A and *vacAm2* genotypes in ulcer patients, while Calcutta (eastern India) reported predominance of *iceA1* and *ice*A2 genotypes, where *ice*A2 was associated with the disease. In Bangladesh, *ice*A2 *H. pylori* genotypes were found in patients with peptic ulcer.[41,42]

Studies from Japan and Korea have reported the prevalence of *cag*A, *vacAs1m1*, and *iceA1* genotypes. But on the contrary, these countries showed no correlation of virulent genotypes with clinical outcomes.[43]

In England, a study found the predominance of *iceA1* alleles, *cag*A, and *vacAs1m2* strains in patients with ulcers. The study also showed genetic affinities of *H. pylori* in England with both European and Asian gene pools.[44]

In Germany, the presence of *H. pylori vacAs1* genotype was reported in 96% of the patients with peptic ulcer disease (PUD), while the *vacAs2* genotype was present in only 4% of these patients, compared to 31% of the patients with non-ulcer dyspepsia.[39]

In African countries like Nigeria, a study showed prevalence of *iceA1*, *cag*A+, and *vacAs1m2* genotypes, and the prevalence of these virulent genotypes was not related to clinical outcomes.[45] In Egypt, *cag*A gene and *vacAs1m1* genotype were associated with gastric cancer patients, whereas *vacAs1m2 H. pylori* genotypes were associated with gastritis cases. The study also found predominance of *ice*A gene in gastric cancer cases compared to cases with gastritis (86.7% vs. 40%).[46]

Much work has also been done on this aspect in Saudi Arabia as well. In the western region, the prevalence of *vacAs1m2*, *vacAs2m2*, and *vacAs1m1* genotypes was 45.6%, 41.8%, and 12.6%, respectively. The study also reported that *vacAs1m2 H. pylori* genotypes were found in gastritis patients (58.6%) and *vacAs1m1* genotypes among patients with peptic ulcers (71.4%).[47,48]

Similarly, the prevalence of *cag*A + and *ice*A genotypes shows significant correlation between *cag*A and the development of gastritis and ulcer cases. This study reported that62.2% of *cag*A and 94.6% of *ice*A2 genotypes were found in gastritis cases. In case of gastric ulcer, the prevalence was 100% for both *cag*A + and *iceA1*.[49] Based on these observations, it can

be concluded that *H. pylori cag*A and *vac*A*s1m2* genotypes are significantly associated with gastritis and peptic ulcer in the Kingdom, although studies from other parts of the Kingdom pertaining to *H. pylori* genotypes are needed to validate this observation.

H. pylori shows genetic diversity in Saudi Arabia. This genetic diversity is in accordance with the findings of clinical isolates tested from other parts of the world [Table 2]. The reason for the observed genetic diversity may be transmission of such genotypes from foreigners hailing from different countries, or natural selection and random genetic drift may have helped to shape the gene pool of *H. pylori* in Saudi Arabia.

Table 2: *Helicobacter pylori* genotypes and its associated clinical outcomes in different countries

Country	*H. pylori* genotypes	Clinical outcomes
Jordan	ice A2	Gastritis and peptic ulcers
	vacAs2 more frequent than vacAs1	vacAs2M2 corellated with erosion
	vacA s1	Gastritis
USA	Ice A1	Peptic ulcers
India Chennai (South India)	cag A and vacAm2	Ulcer
Calcutta (East India)	ice A2	Disease associated
Bangladesh	iceA2	Peptic ulcer
Japan and Korea	cag A, vacas1cm1, ice A1	Not correlated with disease outcomes
England	ice A1 alleles, cag A and vacA s1 m2	Ulcers
Germany	vacAs1	Peptic ulcer disease
	vacA s2	Non-ulcer dyspepsia
Iraq	CagA VacA	Duodenal ulcers and gastritis
Nigeria	ice A1, cag A+and vac As1m2	not related to clinical outcomes
Egypt	cag A vacAs1m1 iceA	Gastric cancer
	vacAs1m2	Gastritis
Saudi Arabia (western region)	vacAs1m2 vacAs2m2	Gastritis
	vacAs1m1	Peptic ulcers
North (Riyadh)	cag A vacAs1m2	Gastritis and peptic ulcers
	vacAs1m1	Gastritis
	CagA along with Vac As1m1	Peptic ulcer
	cagA	Peptic ulcer Gastritis and ulcer
Western region (2007)	cagA+and iceA1	Gastric ulcer
	iceA1	Peptic ulcer

CURRENT ANTI-*H. PYLORI* REGIMENS IN SAUDI ARABIA

Globally, *H. pylori* infection represents a therapeutic challenge with virtually no regime having achieved 100% eradication. Perhaps determining the optimal drug therapy is effected by prudent use of antibiotics in clinical practice, patient noncompliance, and bioavailability of drug in the lumen. Also a high genetic variability in antibiotic resistance from region to region has further complicated the efficacy of drug regimen [Table 3]. This has emphasized the need to tailor the therapy depending upon the prevalence of antimicrobial resistance on a local scale to improve the success rate of eradication.[19,50,51]

Currently, the most prescribed first-line regimen is triple therapy for a week.[17,50] However, this therapy can only be prescribed in areas with low *H. pylori* resistance toward amoxicillin and clarithromycin. "Rescue" therapy or second-line therapy (bismuth-containing quadruple therapy) is given when the first-line therapy fails to act.[22,52]

Saudi Arabia also witnesses increasing emergence of antibiotic resistance to classical therapies, and therefore, it is recommended that the frequent use of MTZ and clarithromycin in clinical practice should be restricted.[53,54] In keeping with the notion, it is imperative to use combination of different antibiotics such as PPI, amoxicillin, and levofloxacin, or PPI, amoxicillin, and tetracycline as first-line therapy in Saudi Arabia, given the fact that studies from other countries also favor the use of levofloxacin-based regimes to counter increasing resistance to clarithromycin and MTZ.[18,52]

EPIDEMIOLOGY OF *H. PYLORI* RESISTANCE IN SAUDI ARABIA

Over the past few years, antimicrobial resistance has emerged in all kinds of micro-organisms worldwide, including Saudi

Table 3: Prevalence of antibiotic resistance rates towards *Helicobacter pylori* in different geographical area

Country	*H. pylori* Antibiotic resistance percentage		
	MTZ	AML	CLR
Saudi Arabia	80	1	4
Western region of Saudi Arabia	48	-	28
North (Riyadh)	69.5	0	21
France	61	-	26
Bangladesh	77	-	15
USA	20-40	-	10-15
Ireland	31	-	33
India	80	33	45
Pakistan	84	37	36

Arabia. This phenomenon has dramatically changed the therapeutic management of diseases including *H. pylori*–associated gastroduodenal problems. For example, a pilot study conducted from 1987 to 1988 and from 1990 to 1996 shows significantly high increase in MTZ resistance in Saudi Arabia from 35.2% to 78.5%. This study has also shown that isolates from females have higher resistance rate to MTZ than those from males.[54] Surprisingly, the rate of resistance to MTZ remained fairly constant till 2008; it was 69.5% for MTZ, while the resistance rates for clarithromycin and amoxicillin were 21% and 0%, respectively.[53] Analysis of available clinical data clearly indicates that the prevalence of MTZ resistance in the entire Kingdom varies from region to region. For example, in the western region of the Kingdom, the rate of resistance to MTZ is 48% and to clarithromycin is 28%.[55] Several reasons have been attributed to the increasing antibiotic resistance to *H. pylori* isolates in Saudi Arabia, for example: 1) frequent usage of drugs in treatment of other bacterial infections such as diarrhea and 2) high prevalence of MTZ resistance in females can be explained as being caused due to its abundant use in gynecological infections, apart from its common usage in the treatment of parasitic diseases.[54] The other reason can be transfer of MTZ-resistant genes from strains harbored by foreigners hailing from India, Pakistan, and Bangladesh where prevalence of MTZ resistance is high. In any case, to overcome the poor patient compliance to MTZ-based antibiotic regimens to *H. pylori* infection, it is recommended to use multidrug regimen. This reaffirms the need of both continuous surveillance for drug resistance and the development of effective prevention and treatment strategies at national and regional levels.

ANTIBIOTIC RESISTANCE MECHANISM

Bacteria develop resistance to various antibiotics which enables them to withstand the harsh environment and multiply. Various mechanisms including mutational inactivation of antibiotic binding site, efflux pump, and horizontal gene transfer have been proposed. For example, resistance to MTZ is primarily associated with mutational inactivation of redox-related genes (*frx*A and *rdx*A).[20,56]

The gene *frx*A may act indirectly by affecting cellular reductive potential in low levels in MTZ-resistant isolates. Alterations of the *rdx*A gene like deletion in the gene and pump efflux system are also of prime importance, but it has not been possible to identify a clear panel of point mutations which could explain the phenomenon. In a nutshell, loss or inactivation of these two genes may lead to MTZ resistance. On the contrary, there are reports suggesting that MTZ resistance phenotype may arise in *H. pylori* without mutations in *rdx*A or *frx*A, suggesting the presence of additional MTZ resistance mechanisms. For example, Choi *et al.* proposed that several mutational changes in *H. pylori* Fur proteins are responsible for differential MTZ susceptibility patterns.[57,58] For clarithromycin, the resistance mechanism is the decreased affinity between ribosomes and clarithromycin. This is due to point mutation in the 23S rRNA gene in 2143 and 2144 positions.[22] In India, emergence of MTZ resistance (Mtz^r) *H. pylori* strains is a result of mutation in chromosomal *rdx*A nitroreductase gene,[42] while in Bangladesh, resistance to MTZ is due to inactivation of *rdx*A gene to confer the Mtz^r phenotype.[59] Saudi Arabia is also witnessing the emergence of *H. pylori*-resistant strains. As discussed earlier, this region shows high prevalence of MTZ-resistant *H. pylori* strains, while resistance to clarithromycin is considerably low. The mechanism for clarithromycin resistance in *H. pylori* from the Kingdom was studied by Bakri, wherein he found 23S rRNA gene to be associated with clarithromycin resistance in *H. pylori*.[60] However, studies on the molecular mechanism for MTZ resistance in *H. pylori* in the Kingdom are yet to be confirmed.

RESISTANCE AND GENOTYPES

Drug resistance and its possible relation with virulence factor genotypes has been studied in some countries. A study in Iran has found the correlation between MTZ resistance and *H. pylori* genotypes. It was found that *cagA*+ and *vacAs1/m2* type was the dominant genotype in Irish *H. pylori* strains. Significant rates of MTZ resistance were observed in *cagA* - group (32%).[50]

A study similar to this reported from Pakistan also showed high prevalence of MTZ and OFX (ofloxacin) resistance in *cagA*- strains as these strains have greater opportunity to acquire antibiotic resistance through mutations. The possible underlying mechanism may be virulent strains causing severe inflammation, thereby increasing the infection, whereas *cagA*- strains acquire mutation more frequently under the selective pressure of MTZ.[61] Contrary to this, it was reported that there is no correlation between clarithromycin resistance and bacterial genotypic pattern and/or *cagA* positivity.[62]

Therefore, genome wide screening is necessary to ascertain that acquisition of antibiotic resistance is a phenomenon for optimizing virulence, especially in the context of non-classical virulence factor present outside the core genome. It will be noteworthy to explore the area of correlation of antibiotic resistance with virulent genotypes in Saudi Arabia as well.

CONCLUSION

This review evidently shows very high prevalence of *H. pylori*-related diseases in Saudi Arabia, although the incidence rate is quite high in adult population as compared to children. However, whether the population

studied was homogenous in nature is not clear. This has bearing on the validities of findings as *H. pylori* is well known for genotypic diversities with regards to geographic locations. Prevalence of *cagA* and *vacAs1m2* genotypes is reported in the Kingdom, but being geographically ethnically different and regionally diverse, it will be more interesting to know about the genotypic diversity and specificity in different ethnic groups of Saudi Arabia. Considerable drug resistance is observed in the KSA, particularly to MTZ, hence alternative regimen with tetracycline, and amoxicillin-or levofloxacin-based regimens may be suggested.

Consistent programs for monitoring drug resistance in *H. pylori* may be initiated. Studies pertaining to resistance and its relation with virulence genotypes in Saudi Arabia may be explored in depth. Also, correlation of circulating genotypes and their potential association with the development of severe form of gastroduodenal diseases including gastric cancer in Saudi Arabia could be noteworthy to explore.

Modern travel system has eased the transmission and spread of infections worldwide. Saudi Arabia harbors a large expatriate population and witnesses a significant number of visitors annually for pilgrimage and/or work from all over the world. These have facilitated the importation of drug-resistant micro-organisms to Saudi Arabia from other countries. Studies considering these aspects will help in developing an effective resistance monitoring program not only for *H. pylori* but also other clinically important micro-organism.

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