Health Research Board

# Application Form

## Summary

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| **Reference** | HRA-POR-2014-526 |
| **Title** | An antimicrobial susceptibility testing-based approach for Helicobacter pylori eradication: will tailored therapy overcome the increasing failure of standard empirical therapy? |
| **Principal Investigator** | Professor Deirdre McNamara |
| **Co-Applicants** | Dr Sinead Smith |
| **Host Institution** | Trinity College Dublin |
| **Duration (months)** | 36 |
| **Budget Total (€)** | 181,570.00 |
| **Abstract** | *Helicobacter pylori* infects approximately half of the world's population. Prevalence of *H. pylori* infection varies globally but increases with older age and with lower socioeconomic status. It remains one of the most common infections in Ireland with infection rates of approximately 22%. *H. pylori* infection is the major cause of peptic ulcer disease and gastric cancers. Eradication of *Helicobacter pylori* is recommended in all symptomatic patients. Standard triple therapy with an acid suppressing proton pump inhibitor, and a dual antibiotic combination with amoxicillin and either metronidazole or clarithromycin remains the first-line treatment. In recent years, treatment success rates have fallen significantly below the 80% recommended by the Maastricht IV Consensus guidelines for the management of *H. pylori* infection. This is in line with a rapid increase in antibiotic resistance, in particular to clarithromycin, with second line and sequential treatments involving levofloxacin, tetracycline or rifabutin often required. The European *Helicobacter* Study Group now recommends that clarithromycin should be abolished from standard anti-*H. pylori* regimens once resistance rates reach 15-20% and have advised local surveillance and monitoring of antibiotic resistance to guide clinicians and improve eradication rates. Through a multicentre and multi-disciplinary approach, this proposal aims to prospectively assess the prevalence of *H. pylori* antibiotic resistance in patient's naïve for *H. pylori* eradication therapy, using standard culture and antimicrobial susceptibility testing methods, as well as recently developed molecular tests. The impact of previous antibiotic use on primary antibiotic resistance will be determined. The efficacy of tailored treatment based on resistance data on *H. pylori* eradication will be compared to that of standard first-line triple therapy, with a view to improving treatment success rates. The expertise we develop will guide clinicians in their therapeutic choice, with a view to achieving improved *H. pylori* eradication rates and thereby better disease prevention and control. |

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## 1. Project Details

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| **Project Title**<br><br>(200 characters limit) | An antimicrobial susceptibility testing-based approach for Helicobacter pylori eradication: will tailored therapy overcome the increasing failure of standard empirical therapy? |

**Project Duration**

Indicate the duration of the proposed project in months. The maximum duration of the award is 36 months.

36

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| **Grant Start Date** | 01/10/2014 |

**Project Lay Summary**

Please provide a Project Lay Summary for the proposed research. This summary may be used when providing information to the public with regards to the variety of research funded by the HRB.

*Helicobacter pylori* infection is a public health issue. It remains one of the most common infections in adults in Ireland (22%) and is associated with significant morbidity and mortality as the primary cause of stomach ulcers and stomach cancers. Infection is more common in lower socioeconomic groups and increases with age. Despite significant work to date resulting in a better understanding of the organism and its complex interaction with man, eradication rates with standard therapies are falling. Primary resistance to commonly employed antibiotics is increasing at an alarming rate, and represents the single largest barrier to the successful management of patients with *Helicobacter pylori*-associated disease. The frequent use of antibiotics such as clarithromycin and metronidazole for common community acquired infections, as well as patient compliance, are major factors which contribute to the decline in treatment success. The wider *H. pylori* research community has recognised the need to address antibiotic resistance as a priority and with some urgency. *Helicobacter pylori* testing for sensitivity to antibiotics is not undertaken routinely in most hospitals and the availability of local primary resistance data is lacking. With a significant track record in *Helicobacter pylori* research, we aim to assess the prevalence of local *H. pylori* antibiotic resistance. To achieve our aims we will analyse stomach samples of a representative cohort of patients who have not undergone eradication therapy previously to test for primary antibiotic resistance. We will assess the impact of tailoring therapy based on antibiotic resistance data with a view to improving *H. pylori* eradication rates. The expertise we develop will assist both community- and hospital-based practitioners through appropriate relevant local feedback on treatments with a view to achieving improved eradication rates and thereby better disease prevention and control.

**Project Abstract**

Please provide a Project Abstract for the proposed research.

*Helicobacter pylori* infects approximately half of the world's population. Prevalence of *H. pylori* infection varies globally but increases with older age and with lower socioeconomic status. It remains one of the most common infections in Ireland with infection rates of approximately 22%. *H. pylori* infection is the major cause of peptic ulcer disease and gastric cancers. Eradication of *Helicobacter pylori* is recommended in all symptomatic patients. Standard triple therapy with an acid suppressing proton pump inhibitor, and a dual antibiotic combination with amoxicillin and either metronidazole or clarithromycin remains the first-line treatment. In recent years, treatment success rates have fallen significantly below the 80% recommended by the Maastricht IV Consensus guidelines for the management of *H. pylori* infection. This is in line with a rapid increase in antibiotic resistance, in particular to clarithromycin, with second line and sequential treatments involving levofloxacin, tetracycline or rifabutin often required. The European *Helicobacter* Study Group now recommends that clarithromycin should be abolished from standard anti-*H. pylori* regimens once resistance rates reach 15-20% and have advised local surveillance and monitoring of antibiotic resistance to guide clinicians and improve eradication rates. Through a multicentre and multi-disciplinary approach, this proposal aims to prospectively assess the prevalence of *H. pylori* antibiotic resistance in patient's naïve for *H. pylori* eradication therapy, using standard culture and antimicrobial susceptibility testing methods, as well as recently developed molecular tests. The impact of previous antibiotic use on primary antibiotic resistance will be determined. The efficacy of tailored treatment based on resistance data on *H. pylori* eradication will be compared to that of standard first-line triple therapy, with a view to improving treatment success rates. The expertise we develop will guide clinicians in their

therapeutic choice, with a view to achieving improved *H. pylori* eradication rates and thereby better disease prevention and control.

**Keywords (Max 5 items)**
Helicobacter pylori

Clarithromycin

Metronidazole

Digestive System Diseases

## 2. Project Description

**Current knowledge, Background to the Area and Relevance**

Describe the background to the research proposal and detail the size and nature of the issue to be addressed.

**Background:** *Helicobacter pylori* specifically colonizes the gastric epithelium and is the most common human bacterial infection worldwide, infecting approximately half of the world's population[1, 2]. Infection is usually acquired in early childhood and persists for decades if left untreated[1, 3]. Prevalence of *H. pylori* varies globally but increases with older age and with lower socioeconomic status. The higher prevalence in older age groups likely reflects poorer childhood living conditions in previous decades[1]. Recent findings from our group indicate that the local prevalence of *H. pylori* infection is 22%[4]. Although most infected patients will not develop any clinically significant complications, *H. pylori* infection confers a 1-10% risk of developing gastric or duodenal ulcers, a 0.1-3% risk of developing gastric adenocarcinoma, and <0.01% of developing mucosa-associated lymphoid tissue (MALT) lymphoma[1]. Gastric cancer is among the top ten most frequently diagnosed malignancies in Ireland, with over 500 new diagnoses annually (National Cancer Registry of Ireland; http://www.ncri.ie/). *H. pylori* has been designated a Class I carcinogen by the World Health Organization[2]. Disease risk in infected individuals is associated with both host genotype and strain-specific bacterial factors.

Consensus guidelines recommend that patients with uncomplicated dyspepsia are managed using a 'test-and-treat' strategy[2]. *H. pylori* infection is diagnosed using non-invasive methods, including the urea breath test (UBT), serologic tests and the stool antigen test[1, 2]. The UBT is commonly employed in the Irish healthcare setting for *H. pylori* diagnosis and involves ingesting $^{13}$C-labelled urea. If present, the *H. pylori* enzyme urease converts the $^{13}$C-labelled urea to labelled carbon dioxide, which is detected in a breath sample. The UBT is rapid, easy to perform and highly accurate with a specificity and sensitivity of 95%[5, 6]. Endoscopy is warranted for dyspeptic patients with accompanying alarm symptoms such as weight loss, persistent vomiting, gastrointestinal bleeding, abdominal mass or iron deficient anaemia [1, 2]. In addition, endoscopy is recommended for patients with new onset dyspepsia above the age of 45[2]. *H. pylori* infection can be detected in endoscopic gastric biopsy samples by several methods. The rapid urease test for *Campylobacter*-like organisms (CLO) involves placing the biopsy specimen in a solution of urea and pH-sensitive dye. The pH change resulting from the *H. pylori* urease-mediated conversion of urea to ammonia results in a colour change indicative of infection[1]. The CLO test has a sensitivity of greater than 90% and a specificity of more than 95%[5]. Histology on the biopsy specimen will also detect the presence of *H. pylori* infection and the degree of inflammation[1, 2]. Culturing of the bacteria from biopsy samples is possible and has the added advantage of allowing for antimicrobial susceptibility testing.

Treatment for *H. pylori* is recommended in all symptomatic infected individuals. Eradication of *H. pylori* provides a long-term cure for both duodenal and gastric ulcers in the majority of patients whose ulcers are not associated with non-steroidal anti-inflammatory drug use[7, 8]. In addition, evidence suggests that *H. pylori* eradication reduces the development of atrophic gastritis and the risk of cancer progression in infected individuals without premalignant gastric lesions[9]. Furthermore, eradication leads to regression of most localized gastric MALT lymphomas[10]. The standard empirical triple therapy for *H. pylori* proposed at the first Maastricht conference on the management of *H. pylori* infection[11] has become widely used throughout the world[2, 12, 13]. This first-line therapy consists of a proton pump inhibitor (PPI) with the antibiotics clarithromycin and amoxicillin taken twice daily for 7-14 days[2]. Metronidazole is used instead of amoxicillin in patients with a penicillin allergy[1].

**The problem:** The success rate of first-line triple therapy has fallen significantly below the recommended 80% in recent years[2, 14], in line with the rapid emergence of antibiotic resistant strains of *H. pylori*[14, 15]. Resistance to clarithromycin is the single biggest cause of treatment failure, and decreases the efficacy of clarithromycin-amoxicillin-PPI triple therapy by up to 70%[16]. As a result, standard empirical triple therapy is now only recommended in regions where clarithromycin resistance is known to be less than 15-20%[2]. While no new drug has been developed as an alternative to the standard triple therapy, recent studies have described the advantages of using different combinations of known antibiotics or extended treatment durations. In regions where clarithromycin resistance is greater than 15-20%, bismuth quadruple therapy (PPI, bismuth salt, tetracycline, metronidazole) is recommended[2]. As bismuth salts are not available in Ireland, non-bismuth quadruple therapies, namely sequential therapy (5 day PPI, amoxicillin; 5 day PPI, clarithromycin, metronidazole) or concomitant therapy (PPI, amoxicillin, metronidazole, clarithromycin) may be prescribed[2]. Following treatment, eradication of *H. pylori* should be confirmed by the UBT or by endoscopy if required. Initial treatment failures have prompted clinicians to prescribe second or third-line antimicrobial regimens that include levofloxacin, tetracycline, or rifabutin[2, 17, 18]. The increasing complexity of these regimens is often associated with a reduction in compliance[19] and an increased risk of side effects.

As the most recent consensus guidelines recommend that clarithromycin should be abandoned if resistance

rates are above 15-20%[2], surveillance of primary antibiotic resistance is warranted to guide clinicians in their choice of therapy. The European *Helicobacter* Study Group (EHSG) have recently reported on the prevalence of primary *H. pylori* antibiotic resistance using a multicentre approach with standardised protocols[15]. The overall resistance rates for clarithromycin, levofloxacin and metronidazole were 17.5%, 14.1%, and 34.9% respectively, with a prevalence ≤1% for tetracycline, rifampicin and amoxicillin[15]. The rate of clarithromycin resistance had almost doubled since the previous European survey[20]. Prevalence of levofloxacin resistance was not tested in the previous European study[20] as levofloxacin-based treatment was introduced later, but several studies have shown an emergence in levofloxacin resistance in the last decade[15, 21, 22]. Metronidazole resistance was high at 34.9%[15] but the rate was similar to that of the previous Europe-wide study[20]. The impact on metronidazole resistance on *H. pylori* eradication is less than that of clarithromycin resistance, and can be overcome by increasing the dose and duration of treatment[14].

The EHSG study also indicated variations in the prevalence of resistance across European countries; the resistance rate for clarithromycin was <10% in Northern European countries, while most countries in the rest of Europe (except Spain and Germany) had a resistance rate of >15%[15]. Recent work from our group has shown a significant increase in the rate of primary clarithromycin resistance locally from 3.9% in 1997 to 9.3% in 2008[23]. The prevalence of metronidazole resistance was 32%[23] and levofloxacin resistance was 11.7%[22]. Despite these worrying trends, antimicrobial susceptibility testing for *H. pylori* is only routinely performed on a weekly basis in two microbiology laboratories in the UK[24]. There are currently no centres in Ireland performing routine resistance testing and monitoring. The lack of antimicrobial susceptibility testing for *H. pylori* needs to be urgently addressed to assist clinicians in prescribing the most effective treatments for *H. pylori* eradication. Indeed, a recent meta-analysis has shown that tailored treatments based on resistance data show better eradication rates and are more cost effective than standard triple therapy in other countries[25]. As antibiotic resistance is a constantly evolving process and there are significant regional variations in resistance rates, the timely surveillance of resistance prevalence locally will allow for tailored recommendations for first-line treatments, with the potential to improve eradication rates and reduce the burden of *H. pylori*-associated disease.

**Overall Aim**

Please state the overall aim of the research project

Using a multidisciplinary approach involving gastroenterologists, scientists and a statistician, this study will prospectively assess the rates of primary *H. pylori* antibiotic resistance using standard culture and antimicrobial susceptibility tests recommended by the EHSG. The effect of previous antibiotic use on resistance rates will be assessed. As culture of *H. pylori* is time-consuming and not always successful, antibiotic resistance will also be tested using recently developed molecular genetic assays. This study will determine whether tailored therapy based on resistance data improves *H. pylori* eradication compared to standard triple therapy, together with the impact of genetic antibiotic resistance on treatment outcome.

**OBJECTIVE 1**

**State Objective**

To assess the primary rates of resistance to the 6 commonly prescribed antibiotics in *H. pylori* strains isolated from endoscopy patients attending 3 Dublin-based hospital sites using standard culture and phenotypic antimicrobial susceptibility testing methods.

**State deliverables in bullet point format**

- These studies will provide data on the local prevalence of *H. pylori* strains resistant to the antibiotics clarithromycin, metronidazole, amoxicillin, levofloxacin, tetracycline and rifabutin.
- As the most recent consensus guidelines recommend that clarithromycin should be abandoned in standard first-line triple therapy if resistance rates are above 15-20%[2], this information will be valuable in the provision of local guidance to both General Practitioners and hospital-based clinicians on the management of *H. pylori* infection.

**OBJECTIVE 2**

**State Objective**

To assess the rates of primary resistance to clarithromycin and levofloxacin using tissue biopsy samples from endoscopy patients attending 3 Dublin-based hospital sites using recently developed molecular genetic tests to detect resistance-associated DNA mutations.

**State deliverables in bullet point format**

- The findings from these studies will inform on the prevalence of primary genetic clarithromycin and levofloxacin resistance in the local catchment area.
- Insight into the concordance between recently developed genetic tests and standard culture and susceptibility testing will be gained.

**OBJECTIVE 3**

**State Objective**

To evaluate the impact of age, gender, ethnicity, endoscopic findings and previous antibiotic exposure on the prevalence of primary *H. pylori* antibiotic resistance.

**State deliverables in bullet point format**

- Potential risk factors associated with harbouring antibiotic resistant strains of *H. pylori* will be identified.
- Identification of risk factors will be useful in predicting antibiotic resistance and potential treatment failure in cases where antimicrobial susceptibility testing or resistance data are not available.

**OBJECTIVE 4**

**State Objective**

To determine whether tailored therapy based on antimicrobial susceptibility testing has a higher eradication rate than standard triple therapy in the treatment of primary *H. pylori* infection.

**State deliverables in bullet point format**

- Information on eradication rates for standard triple therapy versus tailored therapy will be obtained.
- The expertise we develop will assist both community- and hospital-based practitioners through appropriate relevant local feedback on treatments, with a view to achieving acceptable response rates and thereby better disease prevention and control.
- Data acquired will be extremely beneficial in informing how any future National *H. pylori* antibiotic resistance surveillance system could be set up.

**OBJECTIVE 5**

**State Objective**

To determine the impact of both phenotypic resistance and molecular genetic antibiotic resistance on *H. pylori* eradication rates.

**State deliverables in bullet point format**

- The impact of harbouring antibiotic resistant *H. pylori,* as detected by standard culture and sensitivity testing, on the efficacy of eradication therapy in patients treated with standard first-line triple therapy will be assessed
- The impact of genetic antibiotic resistance, as determined by molecular tests, on the efficacy of eradication therapy in patients treated with standard first-line triple therapy will be assessed.
- Taken together, the findings will provide insight into the clinical validity of molecular genetic testing methods, which have the potential to detect *H. pylori* in biopsy tissue samples and identify resistance-associated genetic elements within a significantly more rapid time-frame than standard culture and susceptibility testing (1-2 days versus 1-2 weeks respectively).

(Objectives & Deliverables Support File - Gantt Chart_SS.pdf) is included as an appendix within this file.

**Research Design and Methodological approach**

Summarise the proposed research plan, providing descriptions of individual project/work streams or work packages and describe how they integrate to form a coherent research proposal.

**Patient identification and recruitment.**
Ethical approval will be sought from the Joint Research Ethics Committee of Trinity College Dublin, the Adelaide and Meath Hospital (AMNCH) and St. James's Hospital to cover all the hospital sites in the study. Once ethical approval is in place, patients over the age of 18 years scheduled to undergo endoscopy as a routine part of their care will be invited to participate in the study. Patients will be identified from the endoscopy booking system by principal investigator Prof Deirdre McNamara (AMNCH), in collaboration with Prof Colm O'Morain (Charlemont Clinic) and Prof Nasir Mahmud (St. James's Hospital), all of whom have a significant track record in clinical and translational gastroenterology and *H. pylori* research. It will be explained that the study is for research purposes and potential participants will receive a Patient Information Sheet and consultation prior to committing to the study. Participants will be informed of their right to refuse to participate and of their right to withdraw from the study at any point. This will be stated in the Patient Information Sheet and explained by the staff member seeking consent. Participants will be also be given the opportunity to ask questions in relation to the research study. Patients willing to participate in the study will sign a consent form. Participants will be given a copy of the Patient Information Sheet and signed Consent Form to take with them and an additional copy will be placed in their hospital file. A contact person and phone number will be provided in the Patient information Sheet, should the participant have any queries at a later stage. Should the participant withdraw from the study, any identifiable information will be destroyed and biological samples will be disposed of appropriately.

Exclusion criteria include an inability to give informed consent, severe inter-current illness, pregnancy, breast feeding, coagulopathy, current use of dual anti-platelet therapy, warfarin or allergy to any of the study medication. As is standard for best endoscopy practice, patients will be asked to avoid antibiotics for four weeks and a proton pump inhibitor for two weeks prior to endoscopy, as these agents supress infection and reduce the sensitivity of *H. pylori* diagnostic tests[2]. Study participants will then undergo endoscopy as scheduled and in accordance with local operating protocols. At the time of endoscopy 3 biopsies will be taken from the antrum, one each for histology, the CLO-test and antimicrobial susceptibility testing. A further biopsy will be taken from the corpus for histology. Although not always performed routinely this biopsy regimen is in keeping with international guidelines for identification and appropriate classification of *H. pylori* infection[26].

A positive CLO-test at one hour will be used to identify *H. pylori*-positive patients whose samples will be processed for antimicrobial susceptibility testing and further participation in the study. Patient details including age, gender, ethnicity, key endoscopy findings, previous antibiotic exposure and previous *H. pylori* treatment will be recorded in a case report file. Should participants not have information regarding their previous antibiotic use or *H. pylori* treatment, we will contact their General Practitioner and Pharmacist with their permission. Patients without infection will be informed of their results, released from the study and will resume on-going care from their medical team.

**Data and Statistical Analysis.**
*Population:* Approximately 3,500 gastroscopies a year are performed at the 3 test centres. The estimated local *H. pylori* prevalence is 22%[4]. It is expected that this rate will be somewhat higher (approximately 30%) in the symptomatic patients referred for endoscopy. Thus, there are potentially 1050 infected subjects who could participate in the study. The positivity of the CLO test is approximately 50 % at one hour when the decision to send the sample for antimicrobial susceptibility testing is made[24], and considering a study uptake of 50%, we would aim to recruit approximately 263 subjects per year.

*Power calculation:* The minimum number for each treatment group was determined for a power of 85%, and an alpha of 5%. It was decided that a 10% increase in the eradication rate was considered a significant clinical difference. The baseline eradication rate for the standard triple therapy was taken to be 70%[14]. To be able to detect a 10% increase in eradication rates for the tailored therapy compared to the standard triple therapy, a minimum of 270 patients would will be required in each arm of the study.

*Duration:* To accurately assess the impact of tailored treatment on clinical outcome compared to standard triple therapy, we plan to recruit over a 30 month period to allow for a total population of 660 (330 patients in each group). Follow up and analysis will take a further 6 months. The total study duration will be 36 months.

**Objective 1: *H. pylori* culture and phenotypic antimicrobial susceptibility testing.**
We will use standardised *H. pylori* culture and susceptibility testing methods agreed by the EHSG[15, 24]. These methods are currently in place at the Gastrointestinal Bacteria Reference Laboratory, Public Health England, UK[24]. Training in all of these techniques was provided to co-applicant Dr Sinead Smith during a visit to the Reference Laboratory in March 2013. The biopsy for culture and antimicrobial susceptibility testing will be placed immediately in vial containing 2 ml Dent *Helicobacter* transport medium (brain heart infusion broth containing 2.5% (w/v) yeast extract, 5% sterile horse serum and Dent *Helicobacter* Selective Supplement; Oxoid, Basingstoke, UK)[24] and transported by hand to the Trinity research laboratory for processing. Biopsies will be cultured without homogenisation onto Columbia blood agar plates containing Dent *Helicobacter* selective supplement (Biomerieux, Basingstoke, UK). Following inoculation, biopsies will be stored at -20°C until required

for molecular testing. Plates will be incubated micro-aerobically (86% $N_2$, 4% $O_2$, 5% $CO_2$ and 5% $H_2$) for up to 10 days at 37 °C[24]. Confirmation of *H. pylori* growth will be assessed by the Gram stain, the oxidase, urease and catalase tests.

Fresh cultures (48-72 hours growth) will be used to inoculate minimum recovery diluent broth (Oxoid) to an inoculum concentration of McFarland 3 ($10^8$ colony forming units/ml) and the bacterial suspension used to inoculate Mueller Hinton blood agar plates (Oxoid)[24]. Antimicrobial susceptibility testing for clarithromycin, amoxicillin, metronidazole, levofloxacin, tetracycline and rifabutin will be carried out using Etests (Biomerieux), which are plastic strips calibrated with a predefined concentration gradient of antibiotic and enable the quantitative determination of the minimum inhibitory concentration (MIC) of an antimicrobial agent required to inhibit bacterial growth. The MIC can be read directly from the scale printed on the strip at the point where the edge of the inhibition ellipse of bacterial culture intersects with the strip. Although rifabutin is used clinically, rifabutin Etests are not available in Europe. Rifampicin, which is structurally related to rifabutin and shares many of it's pharmacological properties, is used to screen for rifabutin resistance[24].

An Etest strip for each antimicrobial agent will be applied to an inoculated plate and the plates will be incubated at 37°C under micro-aerobic conditions for 48–72 hours until the inhibition ellipse is clearly visible. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) have defined the clinical breakpoints for antimicrobial resistance to *H. pylori* (Table 1; adapted from EUCAST; www.eucast.org). Double populations and/or isolated colonies growing inside the inhibition ellipse will be considered as evidence of resistance. The MICs of two reference strains will be tested at the beginning of the study and twice during its duration to validate the process. These strains include CCUG 38771 (susceptible to amoxicillin, clarithromycin and metronidazole) and CCUG 38772 (susceptible to amoxicillin, but resistant to clarithromycin >1 mg/L and metronidazole >32 mg/L). Each fresh batch of media will be tested with the control typed strain NCTC 11637. These studies will assess the prevalence of *H. pylori* strains resistant to clarithromycin, amoxicillin, metronidazole, levofloxacin, tetracycline and rifabutin, and the impact of resistance on the eradication rates of standard triple therapy will be evaluated (see Objective 5).

| Antibiotic | Susceptible (mg/L) | Resistant (mg/L) |
|---|---|---|
| Amoxicillin | ≤0.12 | >0.12 |
| Clarithromycin | ≤0.25 | >0.5 |
| Metronidazole | ≤8 | >8 |
| Levofloxacin | ≤1 | >1 |
| Rifampicin* | ≤1 | >1 |
| Tetracycline | ≤1 | >1 |

**Table 1: Proposed clinical antimicrobial breakpoints for *H. pylori***

**Objective 2: Molecular testing for resistance-associated mutations.**

While *H. pylori* culture allows a phenotypic evaluation of antibiotic resistance irrespective of the intrinsic mechanism involved, *H. pylori* is a fastidious bacterium and culture is time-consuming and often difficult with sensitivity values of culture as low as 55-73%[27]. Molecular testing for *H. pylori* offers a complementary approach to culture and allows for molecular genetic identification of *H. pylori* directly from biopsy samples. As such, it provides the opportunity for rapid analysis, enabling same-day diagnosis. Molecular testing has been recommended to detect *H. pylori* and both clarithromycin and quinolone resistance when standard culture and susceptibility testing are not possible[2]. Co-applicant Dr Sinead Smith has significant experience in molecular techniques having completed a BSc in Cell Biology and Molecular Genetics (University College Dublin), a PhD in Cell and Molecular Biology (Dublin City University), and several years of *H. pylori* infection and immunology postdoctoral research employing molecular methods (Trinity College Dublin and Hospital for Special Surgery/Weill Cornell Medical School, USA).

The genetic mutations conferring resistance to clarithromycin and quinolones have been well characterised[27]. Single point mutations within the *H. pylori rrl* gene encoding the 23S ribosomal RNA result in clarithromycin resistance, with three major mutations described; A2146C, A2146G and A2147G (Genbank Accession number NC_000915; formerly described as A2142C, A2142G and A2143G)[27-29]. The most significant mutations conferring quinolone resistance lie at positions 87 and 91 of the *H. pylori gyrA* gene, which encodes the A subunit of the DNA gyrase enzyme involved in regulating the topological state of bacterial DNA during replication[27, 29]. The recently developed GenoType HelicoDR assay (Hain Lifescience, Nehren, Germany) enables detection of *H. pylori* and identification of the resistance-conferring mutations described above. The assay is highly accurate in detecting minimal traces of antibiotic resistant *H. pylori* strains, even in small tissue samples with a sensitivity and specificity of 94% and 99% respectively for clarithromycin resistance and 87% and

98.5% respectively for quinolone resistance[29].

Biopsy tissue samples will first be digested with proteinase K for 4 hours at 56°C and total DNA will be extracted from the samples using the QiaAmp DNA Mini Kit (Qiagen, UK). *H. pylori* DNA sequences will be amplified using a multiplex primer mix (GenoType HelicoDR kit) and HotStar Taq Plus DNA polymerase (Qiagen). The amplified DNA will be hybridized against probes that detect specific *H. pylori* wild-type or mutated elements using GenoType HelicoDR hybridization strips. The presence of bands corresponding to wild type or mutated *H. pylori* sequences that confer clarithromycin of levofloxacin resistance will be assessed using the GenoType HelicoDR evaluation sheet. The presence of wild type and mutated *H. pylori* DNA sequences on the same hybridization strip will indicate the co-existence of *H. pylori* strains susceptible and resistant to the same antibiotic within the same patient sample (hetero-resistance). This is important given that a recent study in relation to genetic clarithromycin resistance indicated that *H. pylori* infection was cured less frequently in patients with pure resistant strains (46%) than those infected with hetero-resistant strains (78.5%) or susceptible strains 94.5%[28]. Molecular tests performed in the proposed study will indicate the prevalence of *H. pylori* strains bearing mutations associated with resistance to clarithromycin and/or quinolones in our local patient cohort. As there is limited data on the effect of genetic antibiotic resistance on *H. pylori* eradication, the impact of these mutations on the outcome of treatment with standard triple therapy will be assessed (see Objective 5).

**Objective 3: Risk factors associated with primary *H. pylori* antibiotic resistance.**
In collaboration with Associate Professor Myra O' Regan (Department of Statistics, TCD), a logistic regression model will be used to determine the association between age, gender, ethnicity, endoscopic findings and previous antibiotic use, and the presence of antibiotic resistance[15, 24, 28]. These findings will identify risk factors associated with harbouring antibiotic resistant strains of *H. pylori* that may be useful in predicting antibiotic resistance and potential treatment failure in cases where antimicrobial susceptibility testing or resistance data are not available.

**Objective 4: Comparison of standard triple therapy versus tailored therapy for the eradication of *H. pylori.***
*H. pylori*-positive patients who have not previously been treated for *H. pylori* infection will be randomised using computer generated randomization software to receive either an empirical course of standard first-line triple therapy or tailored treatment based on their antibiotic sensitivity profile following antimicrobial susceptibility testing (Table 2). To enhance compliance, patients will be given a written synopsis of the daily drug regimen and instructed to carefully adhere to the treatment.

| **Standard 1st line Triple Therapy (7 days)** | **Tailored Therapy (7 days)** |
|---|---|
| Omeprazole (or equivalent) 20 mg twice daily<br>Amoxicillin 1 mg twice daily<br>Clarithromycin 500 mg twice daily | Omeprazole (or equivalent) 20 mg twice daily<br>2 antibiotics from the following:<br>Amoxicillin 1 mg twice daily<br>Clarithromycin 500 mg twice daily<br>Metronidazole 400 mg twice daily<br>Levofloxacin 250 mg twice daily<br>Tetracycline 500 mg four times daily<br>Rifubutin 150 mg twice daily |

**Table 2: Description of treatment regimens for *H. pylori* infection**

All subjects will undergo a routine UBT and follow-up consultation 8 weeks post-treatment to assess *H. pylori* eradication. The UBT will be performed following an overnight fast. A baseline breath sample will be obtained, followed by administration of a drink containing 75mg $^{13}$C-labelled urea with 1.5 g citric acid. A second breath sample will be collected 30 minutes later and the results of the test considered positive if the difference between the baseline sample and the 30 minute sample exceeds 4.0 parts per 1000 of $^{13}CO_2$. During the follow-up consultation, compliance will be assessed and considered good if more than 90% of the drugs are taken. Any subjects with persistent infection after one course of treatment will receive a second line treatment based on their *H. pylori* sensitivity profile and further treatment thereafter as directed by their consultant. In collaboration with statistician Dr Myra O'Regan, eradication rates and their confidence intervals will be calculated by (i) including all the patients involved in the study using intention to treat analysis (ITT) and by (ii) per-protocol (PP) analysis including patients who completed 90% of their medications[30].

**Objective 5: Impact of antibiotic resistance on *H. pylori* eradication rates.**
The impact of harbouring antibiotic resistant *H. pylori*, as assessed both phenotypically (Objective 1) and genotypically (Objective 2), on the efficacy of eradication therapy in the patient group treated with standard first-

line triple therapy will be assessed using a logistic regression model[28] in collaboration with Dr Myra O'Regan.

| **Does your application include a 'pre-clinical' study?** | No |
|---|---|

**Project Management**

Please describe how the research project will be managed

The principal investigator and Head of Department of Clinical Medicine Prof Deirdre McNamara will be responsible for the day-to-day management and implementation of the project and achieving the research goals outlined in the Gantt chart, through interactions with the co-applicant, collaborators and PhD student. They will be heavily involved in all of the clinical aspects of the proposal with regards to patient identification, patient recruitment, sample collection and patient follow-up. Collaborators Prof O'Morain and Prof Mahmud will also recruit patients and collect samples at different hospital sites. The co-applicant Dr Smith will oversee the scientific laboratory aspects of the proposal through direct supervision and mentoring of the PhD student. Statistical support on data analysis will be provided by collaborator Dr O'Regan. Prof McNamara is the director of the Trinity Academic Gastroenterology Group (TAGG) research centre (http://www.medicine.tcd.ie/tagg/), which constitutes a multidisciplinary research team comprising gastroenterologists and scientists working collaboratively on translational projects in gastrointestinal disease. In association with TAGG, weekly research meetings will be held with the immediate research team to assess the project progress against the specific objectives set out in the proposal. Bi-monthly meetings will be held between the immediate research team and the collaborators for more formal research presentations and assessment of data.

Additionally, there are various systems in place within the School of Medicine and TCD to assist in the implementation and management of the research project. There are numerous research groups within the School with complementary programmes providing research support and access to a wealth of knowledge and expertise that will aid the proposed project. In relation to the financial side of the grant, a great deal of interaction will occur with the TCD Treasurer's Office, which handles research contracts, invoicing and produces the financial figures and cost statements for reporting to funding agencies. The TCD Human Resources department will provide information and assistance in relation to human resources policies, procedures and practices. The HR department is responsible for hiring researchers, their inclusion on payroll and assistance is provided in securing visas when necessary.

**Patients, User and Stakeholder Involvement**

Please describe if patients and/or users and/or stakeholders have been actively involved in the preparation of this application and/or will be involved in the proposed research. If this is not applicable to your application please explain why.

The establishment of a resistance research hub locally has the potential to significantly improve patient outcomes, through enhanced eradication rates with improved treatment of active infection, the associated symptoms and the prevention of serious long term complications including gastric cancer. The proposed research is in keeping with the emphasis on patient-orientated outcomes and the improvement in patient care through tailored translational research programmes set locally and nationally. The awareness of resistance as the pivotal issue relating to *H. pylori* management at the European level means that the development of a local research hub would put us in an ideal position to avail of future European initiatives for funding of centres of excellence.

Patients will be directly involved in this project. Patients will be identified from the endoscopy booking system at AMNCH, the Charlemont Clinic and St. James's Hospital. It will be explained that the study is for research purposes and potential participants will receive a Patient Information Sheet and consultation prior to committing to the study. Participants will be informed of their right to refuse to participate and of their right to withdraw from the study at any point. Participants will be also be given the opportunity to ask questions in relation to the research study. A contact person and phone number will be provided in the Patient information Sheet, should the participant have any queries at a later stage. As key stake-holders in the research, every effort will be made to create awareness about the research programme and it's findings to the patient community. Through the Irish Platform for Patients' Organisations, Science and Industry (IPPOSI), we aim to provide information to patients organisations about the nature and impact of our research through participation in meetings and presentation of research findings.

In order to promote interactive communication between the general public and the scientific community, a

number of outreach activities will be undertaken.

- Firstly, members of the research team will visit schools to deliver presentations as part of their career guidance programmes.
- Secondly, transition year secondary school students will be invited to participate in 2-week research projects in the TAGG research centre as part of outreach activities run annually by TCD
- Through the TCD Communications Office, local and national newspapers will be made aware of the research articles published on the HRB-funded project. Articles written in a way that can be understood by non-specialists will be published online and in hard copy format to create awareness to the public at large on health care research in Ireland. In particular the Irish Times will be targeted as it publishes a weekly science supplement with updates on recent advances in research (http://www.irishtimes.com/news/science).

These activities will improve the public's understanding of biomedical research and create awareness about the implications for citizens. Engagement with the public through these specific outreach activities will also help researchers gain a better understanding of public interest in science and their concerns.

**Dissemination and Knowledge Exchange Plan**

Include a clear dissemination and knowledge exchange plan to indicate how information will be disseminated during and after your research.

In order for the research findings to have impact, results will be disseminated to the wider gastroenterology research community through the following methods:

- Publication of the research findings in high quality gastroenterology journals.
- Presentation of research at National and International meetings, including the Irish Society for Gastroenterology Annual Meetings, Digestive Disease Week, United European Gastroenterology Week and the European *Helicobacter* Study Group annual meeting.
- Published articles will be publicized in the research news section of the School of Medicine website, the TCD website, the TAGG website and in the School of Medicine Newsletter.
- Published articles will also be promoted using professional networking sites such as LinkedIn (http://www.linkedin.com), upon which TAGG and the research team members have established a sizeable scientific network.

These dissemination methods will be essential not only for creating awareness about the research to gastrointestinal investigators, but also to gastroenterology healthcare professionals and policy makers. It is expected that data on antibiotic resistance rates will be effectively translated to develop new health care policies and practices for the management of *H. pylori* in Ireland in both hospital and community-based settings. Health care policies and practices will be targeted in the form of annual GP seminars hosted at AMNCH that provide updates and practice guidelines on the management of *H. pylori* infection and the recommended first line triple therapies. In addition, this information will be accessible on the AMNCH hospital website. Prof McNamara is also actively collaborating with the EHSG on their project to create a registry on the management of *H. pylori* infection. Through direct input into this *H. pylori* registry, findings from this proposal will contribute the next Maastricht consensus guidelines for clinicians on the management of *H. pylori* at a European level.

**References**

Please list references cited in Project Description.(Maximum 30 References) Please use the 'Add' function to add each reference one by one.

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