TESIS DOCTORAL

SYSTEMATIC REVIEW OF SEQUENTIAL THERAPY VERSUS STANDARD TRIPLE THERAPY FOR *HELICOBACTER PYLORI* ERADICATION

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Título de la tesis

Systematic review of sequential therapy versus standard triple therapy for *Helicobacter pylori* eradication

Revisión sistemática de la terapia secuencial frente a la triple terapia clásica para la erradicación de *Helicobacter pylori*

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TÍTULO AL QUE OPTA
Doctora en Medicina

“Cuando creíamos que teníamos todas las respuestas, de pronto, cambiaron todas las preguntas”
(Mario Benedetti)

A todo aquello que sea causa directa o indirecta de lo que soy y de lo que aprendo

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- Reunión Anual de la Asociación Española de Gastroenterología (AEG): trabajo seleccionado para comunicación oral en Marzo 2011
- Digestive Disease Week (DDW)— American Gastroenterology Association (AGA)
- International Workshop on Helicobacter and related bacteria in chronic digestive inflammation and gastric cancer— European Helicobacter Study Group (EHSG)

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List of abbreviations

Acronym	Full form
AEs	adverse events
H. pylori	*Helicobacter pylori*
ITT	intention-to-treat
NSAID	non-steroidal anti-inflammatory drug
NUD	non-ulcer disease
PP	per protocol
PPI	proton-pump inhibitor
PUD	peptic ulcer disease
RCTs	randomized controlled trials
RUT	rapid urease test
SEQ	sequential therapy
STT	standard triple therapy
UBT	urea breath test

Executive summary

BACKGROUND: Non-bismuth quadruple sequential therapy (SEQ) has been suggested as a new first-line treatment option to replace the standard triple therapy (STT), in which eradication rates have declined to disappointing levels.

AIM: To conduct a meta-analysis of studies comparing SEQ vs. STT for *H. pylori* eradication.

METHODS: *Selection of studies*: randomized controlled trials (RCTs) comparing SEQ (10 days) and STT (at least 7 days) for the eradication of *H. pylori. Search strategy:* bibliographical searches in electronic databases and manual search of abstracts from Congresses were conducted up to May 2013. *Data synthesis:* intention-to-treat (ITT) eradication rate.

RESULTS: 30 RCTs were included with a total of 8,878 patients (4,173 in the SEQ and 4,705 in the STT). The overall analysis showed that SEQ was significantly more effective than STT (84% vs. 74% in the ITT analysis; OR= 2.06; [95%CI= 1.59-2.66]; p<0.001). Results were highly heterogeneous (I^2=78%) and 10 studies were unable to demonstrate differences between therapies. Subgroup analyses suggested that patients with clarithromycin resistance and/or taking esomeprazole-rabeprazole could benefit more from SEQ. However there were no differences when STT lasted 14 days. Although, overall, mean eradication rate with SEQ was over 80%, a tendency towards lower efficacy with this regimen was observed in the more recent studies [weighted linear regression per year -0.02 (-2% per year) in SEQ vs. -0.005 (-0.5% per year) in STT], and in studies performed outside Italy (OR 1.48 vs. 4.09).

CONCLUSION: The meta-analysis demonstrated that SEQ is more effective than STT lasting less than 14 days. Nevertheless, the apparent advantage of sequential treatment seems to be decreasing overtime; therefore further and continuous assessment is needed before a generalized change in all settings is recommended for first line *H. pylori* treatment.

Resumen

ANTECEDENTES: La terapia secuencial se ha sugerido como primera línea de tratamiento en sustitución de la triple terapia clásica, cuya tasa de erradicación ha disminuido hasta alcanzar valores inaceptables.

OBJETIVO: Realizar un metaanálisis de los estudios que comparan la terapia secuencial frente a la triple terapia clásica para la erradicación de *H. pylori*.

MÉTODOS: *Selección de estudios:* Ensayos clínicos aleatorizados que comparan la terapia secuencial (10 días) y la triple terapia clásica (al menos 7 días) para la erradicación de *H. pylori*. *Estrategia de búsqueda:* electrónica y manual hasta Mayo de 2013. *Síntesis de los datos:* tasa de erradicación por intención de tratar.

RESULTADOS: Se han incluido finalmente 30 ensayos clínicos aleatorizados con un total de 8.878 pacientes tratados (4.173 con la terapia secuencial y 4.705 con la triple terapia clásica). El análisis en su conjunto mostró que la terapia secuencial era significativamente más efectiva que la triple terapia clásica (84% vs. 74% en el análisis por intención de tratar; OR=2,06; [I.C.95%= 1,59-2,66]; p<0,001). Los resultados fueron muy heterogéneos (I^2=78%), y 10 estudios no mostraron diferencias significativas entre ambas terapias. Los subanálisis sugieren que en pacientes con cepas resistentes a claritromicina y/o tomando esomeprazol-rabeprazol se obtiene un aún mayor beneficio con la terapia secuencial. No se encontraron diferencias al comparar la terapia secuencial frente a pautas triples de 14 días. Aunque, globalmente, la tasa media de erradicación superó el 80%, se comprobó una tendencia a una menor eficacia con la terapia secuencial en los estudios más recientes [regresión lineal ponderada -0,02 (-2% por año) en la terapia secuencial vs. -0,005 (-0,5% por año) en la triple terapia clásica] y en los realizados fuera de Italia (OR 1,48 vs. 4,09).

CONCLUSIÓN: El presente metaanálisis confirma que la terapia secuencial es más efectiva que la triple terapia clásica con duración inferior a 14 días. No obstante, la ventaja del tratamiento secuencial sobre el estándar parece ir disminuyendo con el tiempo, por lo que se debería realizar un análisis exhaustivo y continuado antes de recomendar un cambio generalizado en la elección del tratamiento erradicador de *H. pylori* de primera línea.

1. Background

1.1. Description of the condition

Helicobacter pylori (*H. pylori*) infects more than 50% of the adult population globally[1] and is known to be associated with a wide range of upper gastrointestinal diseases including gastritis, peptic ulcer disease (PUD) and gastric cancer. The latest Maastricht IV Consensus[2] has strongly recommended *H. pylori* eradication for patients with PUD, mucosa-associated lymphoid tissue (MALT) lymphoma and atrophic gastritis, post-gastric cancer resection, in patients who are first-degree relatives of gastric cancer patients and in patients with preference after consultation with physicians. It is also suggested that *H. pylori* eradication is appropriate for infected patients investigated for non-ulcer dyspepsia (NUD). Treatment of *H. pylori* may also prevent PUD, bleeding or both in naïve users of non-steroidal anti-inflammatory drugs (NSAIDs).[2]

1.2. Description of the intervention

Since 1997, a worldwide panel of experts, the European Helicobacter Study Group (EHSG) has recommended in its consensus conferences a triple therapy comprising a proton-pump inhibitor (PPI) plus two antibiotics used twice daily as first-line *H. pylori* eradication regimen.[2-5] Commonly, clarithromycin is used together with amoxicillin or nitroimidazole (metronidazole or tinidazole).[5, 6]

However, sequential therapy (SEQ), based on a proton-pump inhibitor (PPI) plus amoxicillin twice daily for the first five days followed by PPI plus clarithromycin together with nitroimidazole twice daily for the following five days, has been suggested as a new treatment to replace the standard triple therapy (STT).[7, 8]

1.3. How the intervention might work

The efficacy of STT is inversely related to the bacterial load and higher eradication rates are achieved in those with a low bacterial density in the stomach.[9, 10] It has therefore been suggested that the short initial dual therapy used in SEQ with amoxicillin lowers the bacterial load in the stomach in order to improve the efficacy of the immediately subsequent short course of triple therapy.[11, 12] In other words, it acts as an induction phase that may amplify the efficacy. The first five days of amoxicillin and PPI thus results in a marked reduction of *H. pylori* and even eradication in at least 50% of patients.[11, 13] The second stage of the regimen (clarithromycin and tinidazole) acts to eradicate a rather small residual population of viable organisms.[13]

Moreover, it has been suggested that the initial use of amoxicillin may offer another essential advantage in the eradication of *H. pylori*.[12] It has been found that regimens containing amoxicillin prevent the selection of secondary clarithromycin resistance.[14] The most accepted candidate theory suggests that SEQ might therefore improve eradication rates as the initial phase of treatment with amoxicillin weakens bacterial cell walls, preventing the development of drug efflux channels involved in the reduction of clarithromycin and other drug concentrations inside the bacteria, although this has not yet been demonstrated. This may allow higher concentrations of antibiotics in the cytoplasm during the second phase of treatment that would facilitate the binding of clarithromycin to the ribosomes.

The sequential administration of antibiotics is not generally recommended because of the fear of promoting drug resistance.[15] However, the initial dual phase of the SEQ uses a drug (amoxicillin) that rarely results in resistance, such that the outcomes should be either cure of the infection or a marked reduction in bacterial load, making the presence of a pre-existing small population of resistant organisms less likely.[16]

1.4. Why it is important to do this review

STT is the most commonly used treatment in clinical practice. However, a critical fall in the *H. pylori* eradication rate following this therapy has been observed since the discovery of *H. pylori*.[15] Even in areas where this decrease has not been demonstrated the efficacy of the STT offers suboptimal results.[17]

Two recent double-blind, US multicentre studies both found disappointingly low eradication rates with STT (77%).[18, 19] Two meta-analyses including both together more than 53,000 patients have shown that the ITT cure rate is below 80%.[20, 21] Therefore, the general recommendation of STT as first line treatment has been questioned, even considering unethical its use as comparator in clinical trials. The use of alternative therapy has been recommended in its place.[22]

The sequential regimen is a novel, promising therapeutic approach but eradication efficacy must be confirmed now that the resistance rate for clarithromycin has increased.[23, 24] Almost all studies using the sequential regimen published during 2008, 2009 and 2010 had lower than 90% eradication rates and in some cases lower than 80% figures were reported.[25, 26]

Moreover, the most commonly used SEQ uses tinidazole, whilst in some studies metronidazole has been used. A recent review on SEQ[27] showed that the eradication rate achieved with metronidazole-based regimens was significantly lower than that achieved with a tinidazole-based

regimen. Indeed, tinidazole has a markedly longer half-life compared to metronidazole and this could be cause for concern for successful *H. pylori* therapy.

It is also important to mention that most of the studies considered in the previous pooled analyses and meta-analyses were performed in Italy.[27-29] Some of the more recent studies, including other regions, have not demonstrated a beneficial effect of SEQ when compared with STT but have instead shown equivalent eradication rates.[30, 31] All these issues require an update of previous meta-analyses and the sub-analysis of these factors in order to evaluate the current benefit reached with SEQ over STT, and to measure those variables that may affect the eradication rates.

Previous meta-analyses have compared STT with SEQ.[28-30] In our preliminary search, we identified several randomized controlled trials (RCTs) which were not included in the previous meta-analyses. We therefore conducted a systematic review of RCTs comparing SEQ versus STT for *H. pylori* eradication, using more databases, optimized search strategies and applying the rigorous techniques recommended by the Cochrane Collaboration and including later published studies.

This systematic review was developed from an on-going Cochrane Review of all *H. pylori* eradication therapies.[32]

2. Objectives

2.1. Primary objective

To conduct a meta-analysis of randomized controlled trials comparing the efficacy of a sequential regimen vs. standard triple therapy for the eradication of *H. pylori* infection.

2.2. Secondary objective

To compare the incidence of adverse effects associated with both standard triple and sequential *H. pylori* eradication therapies.

3. Methods

3.1. Criteria for considering studies for this review

This systematic review was conducted using the manual for developing systematic reviews: *The Cochrane Handbook for systematic reviews of Interventions*.[33] Since systematic reviews are by nature retrospective, it is essential to document the methods to be used. A pre-defined approach was therefore developed as part of the protocol[34] prior to knowledge of the evidence available to reduce the impact of review authors' biases, promote transparency of methods and processes, and allow peer review of the planned methods and different stages to guide the systematic review.[35]

The protocol outlined the study question, detailed the criteria against which studies were to be assessed for inclusion and described how the review process should be managed (for instance, by identifying the methods used, the type of information to data extract and how upcoming amendments were to be dealt with). The protocol defined fields such as the health problem, the intervention under investigation, the outcomes measures, and the study design of interest. The process for identifying, evaluating and summarizing the studies across the review was additionally described and validated by several clinical experts.

Any modification taking place during the systematic review process was highlighted and reported within the corresponding phase / heading of present report.

3.2. Selection criteria

3.2.1. Types of studies

Only parallel-group, randomized controlled trials (RCTs) were eligible for inclusion in the review. Non-randomized studies, case reports, letters, editorials, commentaries and reviews were excluded.

Regarding the date of the publication, no threshold was defined. No language restrictions were applied during the eligibility of studies.

3.2.2. Types of participants

The study population included adults and children diagnosed as positive for *H. pylori* (with at least one confirmatory test) on the basis of monoclonal stood antigen test, rapid urease test (RUT), histology or culture of an endoscopic biopsy sample, or by a urea breath test (UBT).

Trials in which participants were diagnosed as *H. pylori* positive solely on the basis of serology or polymerase chain reaction (PCR), or who had previously been treated with an eradication therapy were excluded.

3.2.3. Types of interventions

Trials comparing a SEQ vs. a STT for *H. pylori* eradication as defined below were included. Additionally, studies that were not assessing an *H. pylori* treatment or that were focusing on other gastrointestinal conditions were directly excluded.

Any variations in the interventions' schedule other than the specified regarding its length or the type or dosage of the antibiotic used were subject to the study exclusion.

3.2.3.1. Sequential therapy (SEQ)

The 10-day SEQ was comprised of a PPI twice daily and amoxicillin 1 g twice daily for the first five days followed by PPI twice daily, clarithromycin 500 mg twice daily and tinidazole or metronidazole twice daily for the following five days.

3.2.3.2. Standard triple therapy (STT)

The STT consisted of a PPI twice daily, clarithromycin 500 mg twice daily and amoxicillin 1 g twice daily lasting at least seven days.

3.2.4. Types of outcomes measures

3.2.4.1. Primary outcomes

Trials were included if they reported the number of patients with *H. pylori* eradication.

Trials were eligible if *H. pylori* eradication was confirmed using RUT or histology of an endoscopic biopsy sample or by a UBT or a monoclonal stool antigen test, at least four weeks after completion of treatment.

Assessments by serology test alone or by culture alone were not satisfactory and subject to exclusion.

3.2.4.2. Secondary outcomes

Reported incidence of AEs was also included.

AEs incidence was recorded as the number of patients reporting: any type of AE; any gastrointestinal disturbance such as nausea or vomiting; any dermatological problem; any systemic effect (fever, headache or dizziness); or any serious AE.

A serious AE was assumed as the occurrence of any undesirable and important medical event such as for e.g. death, life- threatening situation, hospitalization, permanent damage associated with any medical drug. A serious AE was not to be confused with a severe AE – an intense form of AE that usually incapacitates individual normal life. Reported severe AEs were also collected.

Reported treatment compliance (or adherence) rate defined as the extent to which a patient fulfilled with the corresponding prescribed treatment in terms of drug type, dosage and length was collected.

Reported withdrawals rate recorded as the number of patients discontinuing treatment due to AEs, was also of interest.

3.3. Search methods for identification of studies

3.3.1. Electronic searches

Bibliographical searches were performed in MEDLINE, EMBASE and CINAHL electronic databases and in the Cochrane Central Register of Controlled Trials (CENTRAL) through the Cochrane Library (Appendix: search strategy).

Search terms were combined to capture 2 components of the study question: the disease (*H. pylori* infection) and the intervention of interest (standard triple therapy vs. sequential therapy). Following combination of terms (all fields) was used: (Helicobacter OR pylori) AND sequential AND (triple OR “standard regimen” OR “standard therapy”). Hand-searches were adapted and performed using the same syntax.

The design of the search was refined by the Trials Search Coordinator at the Cochrane Upper Gastrointestinal and Pancreatic Diseases Review Group of the Cochrane Collaboration.

The first electronic search was run up to February 2011 and 4,670 citations (MEDLINE= 1,365; EMBASE= 3,504; CENTRAL= 1,569; CINAHL= 362) were retrieved. Due to time constraints, an additional search was run up to May 2013 and 125 new citations were added (MEDLINE= 76, EMBASE= 27, CENTRAL= 30, CINAHL=30).

3.3.2. Searching other sources

Additional hand-searches of websites were conducted in order to retrieve additional publications not captured by the electronic searches. The manual search aimed to identify abstracts of RCTs that might not have been published in peer-reviewed journals but only as part of conference proceedings, specialised journals or international congresses such as the International Workshop of the European Helicobacter Study Group (EHSG), the American Digestive Disease Week (DDW) and the United European Gastroenterology Week (UEGW).

Each of the abstracts identified as potentially eligible was reviewed and only those meeting the inclusion criteria were recorded for inclusion.

Detailed cross referencing from bibliographies of the included studies as well as from other systematic reviews was conducted in order to identify further relevant trials.

3.4. Data collection and analysis

3.4.1. Selection of studies

Prior to the selection of studies phase, most duplicates were automatically removed when studies were imported to the citation manager for their management. Remaining duplicates were removed manually during the first screening phase.

The selection of retrieved studies from the searches was conducted in two phases. An initial screening of titles and abstracts (first screening phase) against the inclusion criteria was undertaken to identify the potentially relevant publications. Following this step, the screening of the full paper (second screening phase) of the studies identified as potentially eligible for inclusion during the first screening phase was performed.

In the case of abstracts or articles with insufficient detail to meet the inclusion criteria, authors were contacted.

Based on the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) approach (http://www.prisma-statement.org), a diagram was developed in order to schematize previously mentioned steps used for the identification and the selection of studies. The number of studies considered at each step and the reason for exclusion of each of the excluded studies was specified.

The process of both the first and second screening was carried out by two review authors (AGM and OPN) independently. Any discrepancies were resolved by discussion and a third review author (JPG) was consulted for unresolved disagreements.

3.4.2. Data extraction and management

A data extraction form that had been previously pre-tested was developed during the protocol phase to record data from the selected papers. Following fields were collected during the data extraction process:

- first author's name and year of publication;
- country;
- format of publication (abstract versus journal article);
- age of the population (adult versus children);
- medical condition (PUD or NUD or other);
- number of patients in each treatment group;
- name, dose and timing of antibiotic administration;
- length of STT;
- eradication rate per treatment regimen [intention-to-treat (ITT) and per-protocol (PP)]: if only the PP sample was reported, the ITT sample was calculated on the basis of the randomization and drop-out information;
- definition of compliance and the compliance rate in the ITT sample;
- details of the method of assessment of *H. pylori* infection both before and after treatment;
- whether the antibiotic sensitivity and resistance was tested before and after eradication; if so, the primary and secondary antibiotic resistance rate;
- incidence, type and severity of AEs;
- study quality: generation of the treatment allocation, concealment of the treatment allocation at randomization, implementation of masking, completeness of follow-up and use of ITT analysis.

Authors of primary studies were contacted for any missing data.

The process of the data extraction was undertaken by two review authors (AGM and OPN) independently. Consensus was reached when discrepancies occurred. If consensus was not achieved, the opinion of a third review author (JPG) was obtained.

3.4.3. Assessment of risk of bias in included studies

Four components of quality were assessed following the quality checklist recommended in the *Cochrane Handbook for Systematic Reviews and Interventions*. [33] The quality of the trials was assessed according to the information available in the published trials as per the risk of overestimating intervention effects in RCTs with inadequate methodological quality. [36] Authors were contacted for any missing information. Items assessed are described and listed in the headings below.

Two review authors (AGM and OPN) assessed independently the quality of methodology of all included studies in the review. As in previous phases, the opinion of a third review author (JPG) was obtained in case of disagreement.

3.4.4. Generation of the treatment allocation

A study was considered as a RCT if it was explicitly stated as 'randomized'. This should include the use of words such as 'randomly', 'random' or 'randomization'.

The randomized trial was then judged as 'truly random, pseudo-random, non-random or not stated'.

A trial was defined as 'truly-random' if the allocation sequence was computer-generated or generated by a random number table, coin toss, shuffles or throwing dice. The person involved in the recruitment of participants should not be the one performing the procedure.

If the selection was based on patient numbers, birth dates, visit dates, alternate allocation or other method not involving a defined random mechanism but likely to produce an unpredictable sequence of numbers, the trial was to be considered as 'pseudo-random'.

Studies were the selection was based on patient or clinical preference, or any selection mechanism that could not be described as random, were to be excluded. Also, studies that did not state whether the treatment was randomly allocated were to be excluded.

3.4.5. Concealment of the treatment allocation at randomization

A study was referred as concealed, unconcealed or unclear in the following situations.[37]

A study was considered 'concealed' if the trial lists were unaware of the allocation of each participant before they were entered in the trial. Adequate methods included central telephone randomization schemes, pharmacy-based schemes, sequentially numbered, opaque, sealed

envelopes, sealed envelope from a closed bag, or the use of numbered or coded bottles or containers.

The allocation was ‘unconcealed’ when trial lists were aware of the allocation of each participant before they were entering the trial. For example when it was based on patient data, such as date of birth or hospital case note number, visit dates, sealed envelopes that were not opaque, or a random number table that was not concealed from the investigator.

If authors did not report or provide a description of an allocation concealment approach that allowed for classification as concealed or not concealed then the study was categorized under ‘unclear allocation concealment’.

3.4.6. Implementation of masking

A trial could be considered double-blinded, single-blinded, not blinded or unclear and was to be recorded within a risk of bias table into 3 categories: low risk, unclear risk and high risk.[33]

A study was judged as ‘not blinded’ if the authors defined it as an open-label study. It was flagged as ‘high risk’.

Studies were to be categorized as ‘unclear risk’ if no blinding information was reported.

If a trial was simply described as 'single-blind' the degree of masking was recorded as ‘unclear’ for clinician and outcome assessor when subjects were presumed blinded.

In the case of a trial was reported ‘double-blind’ it had to be flagged as ‘low risk’. Double blinding was however not expected to occur as per the type of treatment administration could not easily allow the blinding of the clinician, the outcome assessor, the participant and the pharmacist at the same time.

3.4.7. Completeness of follow up and use of intention-to-treat (ITT) analysis

The proportion of participants for which there were missing outcome data and/or who were excluded from the analysis were to be noted for each arm of the trial and in the ITT analyses these subjects were assumed to have failed therapy. It was stated whether the analysis included all randomized subjects that is, whether an ITT approach was undertaken.

The authors’ definitions were recorded when they reported an ITT analysis. Due to the varied definitions of ITT by authors, the most accepted definition of the ITT approach was used. All subjects were to be analyzed in the groups to which they were originally randomly assigned, regardless of

whether they satisfied the entry criteria, whether the treatment was received, or subsequent withdrawal or deviation from the protocol was performed.[38]

All available information for all randomized subjects was reported. Studies reporting either ITT or PP analysis alone were included. Authors of those primary studies either using a different ITT approach (as the one used in current meta-analysis) or reporting PP analysis only, were contacted in order to obtain the usual ITT analysis approach.

Studies reporting ITT analysis but required subjects to have the second test confirming their *H. pylori* infection status after randomization in order to be included in the ITT analysis were equally included for the ITT meta-analysis.

These four quality components were taken from the key methodological features that are important to the validity and interpretation of included trials as mentioned above. No quality score was given and studies classified as 'low quality' were not excluded. The individual quality assessment items were to be used to explore heterogeneity if significant heterogeneity between studies was shown (details below), by using subgroup analysis with pooled effect size estimated, and discuss them when interpreting the results.

3.5. Measures of treatment effect

Dichotomous outcomes of individual studies were expressed as the odds ratio (OR) together with the 95% confidence interval (CI), taking *'H. pylori* eradication' as the primary outcome. The OR describes the ratio of the odds of an event, for which a value of 1 indicates that the estimated effects are the same for both interventions.[33]

The sequential treatment arm was labelled as the treatment group and the standard triple therapy arm as the control group.

Meta-analysis was performed combining the ORs of the individual studies in a global OR using a fixed-effect (Mantel-Haenszel) method primarily, with the random-effects (DerSimonian and Laird) model used in addition as a sensitivity analysis to check the robustness of the results.[39, 40] Pooled effect estimations were performed using Review Manager 5.2 (RevMan 2012).[41] All outcomes included in this review were binary. Data analyses were performed for each of the comparisons included.

There are several methods to calculate number needed to treat (NNT) and some have limitations.[42-44] Many published meta-analyses do not provide the results or the methods used. In this report, the NNT was calculated for efficacy and the number needed to harm (NNH) for adverse events.

Initially, the formula specified within the protocol document was NNT = 1/ [(1-RR) * CER][45] where CER was the pooled control event rate. This formula was later modified and adapted to the measure of the effect size utilized in present review, i.e. odds ratio. The formula used was therefore NNT= (1-(CER*(1-OR)))/ ((1-CER)*(CER)*(1-OR)). The NNT was always reported among those statistically significant comparisons, and where the OR was greater.

3.5.1. Unit of analysis issues

Only standard design, parallel, randomized controlled trials were included. The interest was only in the direct comparison between the two treatment regimens. Multiple groups to a single pair-wise comparison were not included, so that the same patient was not used twice in the same analysis.

However, multiple group comparisons are usual across treatment arms in clinical trials. For instance, the ITT population could be randomized into 3 different treatment arms (or schedules): standard triple therapy lasting 7 days, standard triple therapy lasting 14 days and sequential therapy lasting 10 days. In such case, for the purpose of the overall analysis, the different arms of the same treatment (i.e. STT-7days and STT-14 days) were combined by summarizing the number of patients of each arm. Afterwards, the corresponding subgroup meta-analyses were undertaken.

The patient was used as unit of analysis. The different treatment schedules within the same treatment arm were in addition further assessed through standard single pair-wise comparisons as specified under the subgroup analyses section.

3.5.2. Dealing with missing data

Authors were contacted for any incomplete outcome data from included studies. Those patients for whom outcome data were still missing (due to drop-out or incomplete records) were considered as to have failed eradication for the primary outcome.

3.5.3. Assessment of heterogeneity

In order to identify the possible diversity in trial characteristics, the clinical, methodological and statistical components were analyzed.

The Chi^2 test for heterogeneity was carried out for each combined analysis, where $p < 0.10$ indicated significant heterogeneity between studies.[46] The I^2 statistic, which quantifies heterogeneity by calculating the percentage of total variation across studies that is due to heterogeneity (an approach that has recently been endorsed by the Cochrane Collaboration) was reported. Significant heterogeneity was defined as $I^2 > 25\%$ based on the judgment that I^2 values below 25%, 50% and 75% represent low, moderate and high heterogeneity, respectively.[47]

Graphical methods (forest plots) were used to complete the Chi^2 test assessment. When heterogeneity was identified the source was investigated using further techniques, such as subgroup analyses or funnel plots, to work out whether particular characteristics of studies were related to the sizes of the treatment effect, according to the *Cochrane Handbook for Systematic Reviews of Interventions.*[33]

3.5.4. Assessment of reporting biases

To assess publication bias, funnel plot asymmetry was inspected visually by examining the relationship between the treatment effects and the standard error of the estimate. Funnel plots were produced for the principal outcome for each comparison (plots of OR against the standard error (log of OR)).

3.5.5. Data synthesis

In order to collate, combine and summarize the information from the included studies, it was decided to undertake a quantitative (meta-analysis) approach. If there were insufficient trials (≤ 2) reporting for the same comparison, then a qualitative (narrative) evaluation was performed.

As first step for the data synthesis, an initial overview of results was presented referring generally to all included studies (see 4.1.1). These overall findings were presented in a descriptive fashion in terms of geographic region, target populations, sample sizes, age of the population, medical condition at baseline and treatment schedules assessed.

The second step in the evidence synthesis consisted of summarizing the information related to the size of the effect of the intervention for all studies as well as for each different participant group, comparison or outcome measure undertaken. Results from subgroup analyses as well as sensitivity analyses were also reported.

3.5.6. Subgroup analysis and investigation of heterogeneity

Pre-planned, a priori, subgroup analyses were performed regardless of whether significant heterogeneity was present on the subgroups of:

- geographic region;
- publication date;
- age (children versus adults);
- length of standard triple therapy (7 versus 10 versus 14 days);
- type of nitroimidazole (metronidazole versus tinidazole);
- dosing for each antibiotic;
- resistance of each antibiotic;
- PPI type and dosing (new versus old generation);
- type of disease at enrolment (PUD versus NUD);
- other aspects of study quality: methods of randomization, concealment of allocation versus unconcealed/unclear , blinding; and
- publication status (abstract format versus full-article format) which was additionally added as it was not initially stated in the protocol

3.5.7. Sensitivity analysis

No arbitrary inclusion or exclusion criteria were established for the search strategy. If during the review process sensitivity issues were identified (missing data, individual peculiarities of the studies) the meta-analysis was repeated using the different assumptions made.

Sensitivity analyses were performed to test the robustness of the review: using a random-effects model instead of a fixed-effect model; excluding trials with no or unclear allocation concealment; excluding trials where the method of randomization was unclear; or excluding trials where masking was unclear.

4. Results

4.1. Description of studies

4.1.1. Results of the search

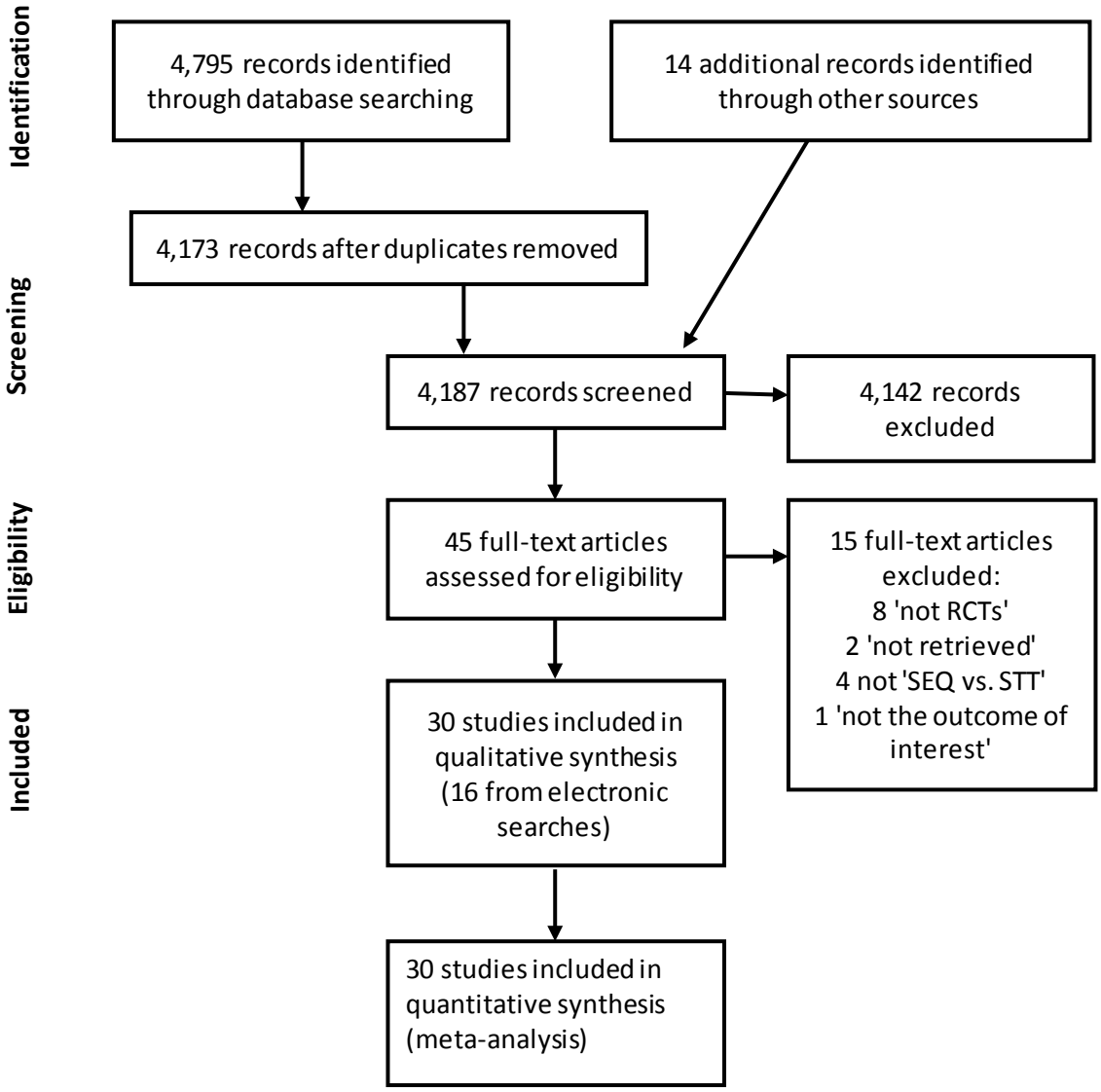
A total of 4,795 citations were retrieved from the following electronic databases: Ovid (Medline), EBM Reviews - Cochrane Central Register of Controlled Trials, CINHAL and Embase, while 14 additional references were identified through hand-searches and from the International Workshop of the European Helicobacter Study Group, the American Digestive Disease Week (DDW) and the United European Gastroenterology Week (UEGW) Congresses up to May 2013.

After removal of duplicates, 4,173 citations resulting from the electronic searches only were initially screened. Based on the review of their corresponding titles and abstracts 4,142 citations were excluded, while 31 full papers were aimed to be retrieved, either because they were potentially relevant, or because not enough information was reported in the title and abstract to make a final decision regarding the inclusion of the paper in the systematic review.

After review of the full papers, 30 publications were finally included in the systematic review: 16 references from the electronic database searches and 14 resulting from the hand-searches. All of them were randomized controlled trials (RCTs).

A description of the process followed for the identification and selection of studies, and the number of studies identified through each step, is presented as part of the PRISMA diagram (Figure 1).

Figure 1. Study flow diagram



4.1.2. Included studies

A total of 30 RCTs with standard parallel group designs were finally included in current systematic review.

The primary objective of almost all the included studies was very similar and aimed to assess the efficacy of the 10 day sequential therapy (SEQ) over the standard triple therapy (STT).

Only 2 references reported different primary objectives: the article published by De Francesco in 2004 (b)[48] aimed to identify predicting factors for the outcome of *H. pylori* eradication using two therapeutic schemes (triple and sequential) and the article by Molina-Infante, 2010[49] whose primary objective was to compare clarithromycin and levofloxacin in triple and sequential first-line regimens.

For the purposes of the evidence synthesis, the included studies were categorized according to the relevant endpoint assessed, i.e. the overall eradication rate with SEQ and STT as well as the different variables evaluated within the subgroup analysis.

Of the included studies, 11 were published in Italy[48, 50-59], 6 in Korea[25, 60-64], 5 in China[65-69], and one each in Iran,[70] Latin-America,[71] Poland,[72] Puerto-Rico,[73] Belgium[74], Morocco[75], India[76] and Spain.[49] Nine of the included studies were published before 2008.

Three studies [69, 72, 74] published between 2010 and 2011 assessed the efficacy of 10-day SEQ versus STT in children.

Eleven studies[48, 49, 56, 58, 59, 61, 62, 68, 70, 71, 76] assessed the efficacy of SEQ versus STT in both NUD and PUD patients´ groups. Cure rates were reported for each of the groups independently and the studies were pooled within the corresponding subgroup analysis.

The sample sizes across the included studies varied considerably ranging from 36 patients within the STT arm and 40 patients within the SEQ arm in the study by Lu, 2010[69] to 522 patients within the SEQ arm and 527 patients in the STT arm in the study by Zullo in 2003.[59]

All studies compared 10-day SEQ versus the STT. The STT included different regimens' lengths (7, 10 and 14-days) and different antibiotic doses (high and standard doses). The SEQ utilized different nitroimidazole types (metronidazole and tinidazole), and both regimens varied the type and dosage

of PPIs: omeprazole, lansoprazole, pantoprazole, rabeprazole, or esomeprazole. One study[54] used double-dose PPI and another[61] low-dose PPI in both treatment arms.

Further details on the different interventions evaluated across included studies are reported in Table 2.

4.1.3. Excluded studies

The total number of studies finally excluded after both the first and second screenings was 4,157.

In almost 50% of the cases, studies were excluded as they were not RCTs and 34% of the studies were discarded as they did not compare the pre-specified therapies (Table 1).

Table 1. Reasons for excluded studies.

Reason for exclusion	Number	%
Not in humans	20	0.42
Abstracts that were reported elsewhere	11	0.23
Not an RCT	2,353	49
Not *H. pylori*	116	2.4
Not SEQ vs. STT	1,651	34
Previous eradication therapy	3	0.06
Not the outcome of interest	1	0.02
Not retrieved*	2	0.04
Total	**4,157**	100

*References could not be accessed

4.2. Effects of intervention

4.2.1. Overall *H. pylori* eradication rate

A total of 30 studies were finally included in the overall analysis comparing SEQ versus STT (summary Table 2).

Note that for the overall analysis, when combining data and only within the STT arm, several studies did randomize patients into up to 3 different STT arms (7, 10 and 14 days). In order to preserve randomisation and weight among included studies, the final proportion of patients cured with STT was calculated into a single figure, by adding the number of patients cured in each of the 3 STT arms

(as stated in the section 'Unit of analysis issues'). Then the total of events (total STT cure rate) was divided over the total of patients assessed within the 3 STT arms.

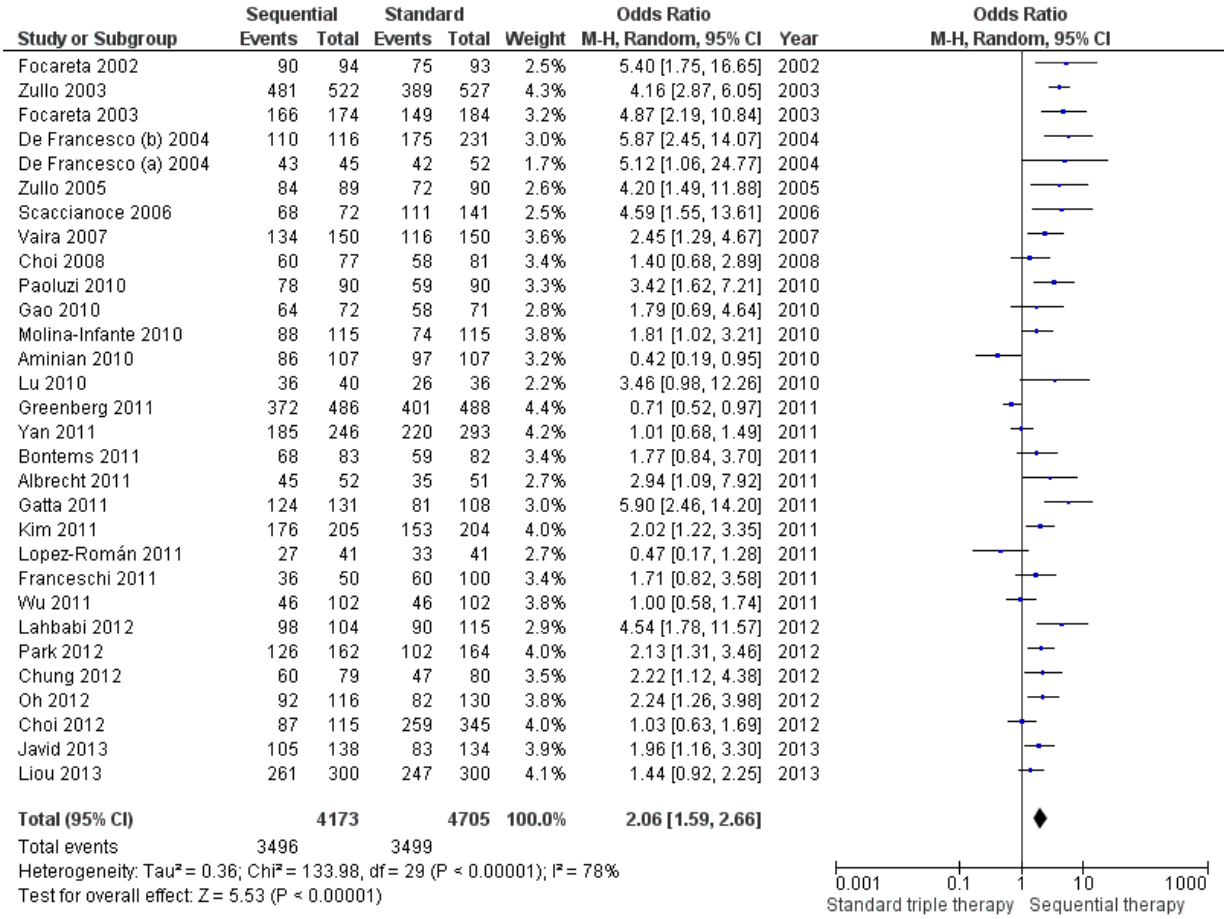
The meta-analysis showed that in the ITT analysis, overall eradication rate was higher with SEQ than with STT (OR= 2.06; [95%CI= 1.59-2.66]; p<0.001; Figure 2). The NNT for the *H. pylori* overall ITT eradication rate was calculated as 9.

Results were highly heterogeneous (I^2= 78%), therefore a random effect model was considered more appropriate to combine the dichotomous outcomes of the different studies.

On the other hand, two studies[70, 71] demonstrated a significantly higher efficacy with STT. Both of the studies assessed adult patients: the study by Aminian et al published in Iran in 2010 reported an ITT cure rate of 91% and 80% with STT and SEQ respectively. Similarly, the study by Greenberg et al published in 2011 as part of a multicentre trial in Latin America reported an ITT cure rate of 82% and 76% with STT and SEQ respectively.

Also, 10 of the included studies could not demonstrate any clinical benefit from one regimen over the other. Seven of the 10 aforementioned studies were performed in Asia (China and Korea), 1 in Puerto Rico,[73] another[74] was published as part of a multicentre trial involving Western European countries (France, Belgium and Italy) and another study[53] was performed in Italy.

Figure 2. Forest plot of comparison: SEQ vs. STT, overall outcome: Eradication rate



H. pylori eradication rate with SEQ ranged from 45% in the Chinese study by Wu performed in 2011[66] to 96% in the Italian study by Focareta in 2002[51] (Table 2).

The trend-line, expressed as the polynomial function of both SEQ and STT eradication rates showed how SEQ cure rates fluctuated before and after 2008 (Figure 3). An order 4 polynomial trend-line (4 bends) has been used to illustrate the relationship between SEQ cure rates and the year of the publication of the studies. The polynomial function of SEQ was defined by: y= -0.0004x4 + 0.0273x3 - 0.5431x2 + 2.3047x + 92.793; where R^2= 0.385 showing an acceptable fit of the line to the data. The valley in the curve of SEQ shows a decrease in eradication rates below 90% from year 2008 but for 3 studies which cure rates were greater or equal to 90%.[54, 69, 75]

Equally, an order 4 polynomial trend-line was chosen to illustrate the trend in eradication rates with STT through the time and to compare such curve with SEQ trend-line. The polynomial function of STT was defined by: y = 0.0003x4 - 0.0178x3 + 0.3028x2 - 2.3484x + 83.24; where R^2= 0.2015. As shown

in the graph (Figure 3), the absolute difference in eradication rates between SEQ and STT are lower between years 2010 and 2011.

Similarly the linear weighted regression chart (Figure 4) presented the same tendency into a lower efficacy through the years in the overall mean eradication rate for both therapies. As stated, the regression is controlled by each study weight so that both spatial trends and spatial autocorrelation between eradication rates is accounted within the usual statistical assumptions of regression. A stronger trend from the same year 2008 onwards was highlighted.

Moreover, cure rates with SEQ appeared constant and continuous among the Italian studies published before 2008 whereas this un-interrupted level of eradication achieved ceased from year 2008 (Figure 5).

In total, eleven (37%) studies performed in Italy showed that ITT eradication rates with SEQ were over 90%; whereas in the remaining studies ITT rates were mostly below this threshold (Figure 3 and Figure 6).

Figure 3. Efficacy of 10-day SEQ vs. STT by included study and publication date

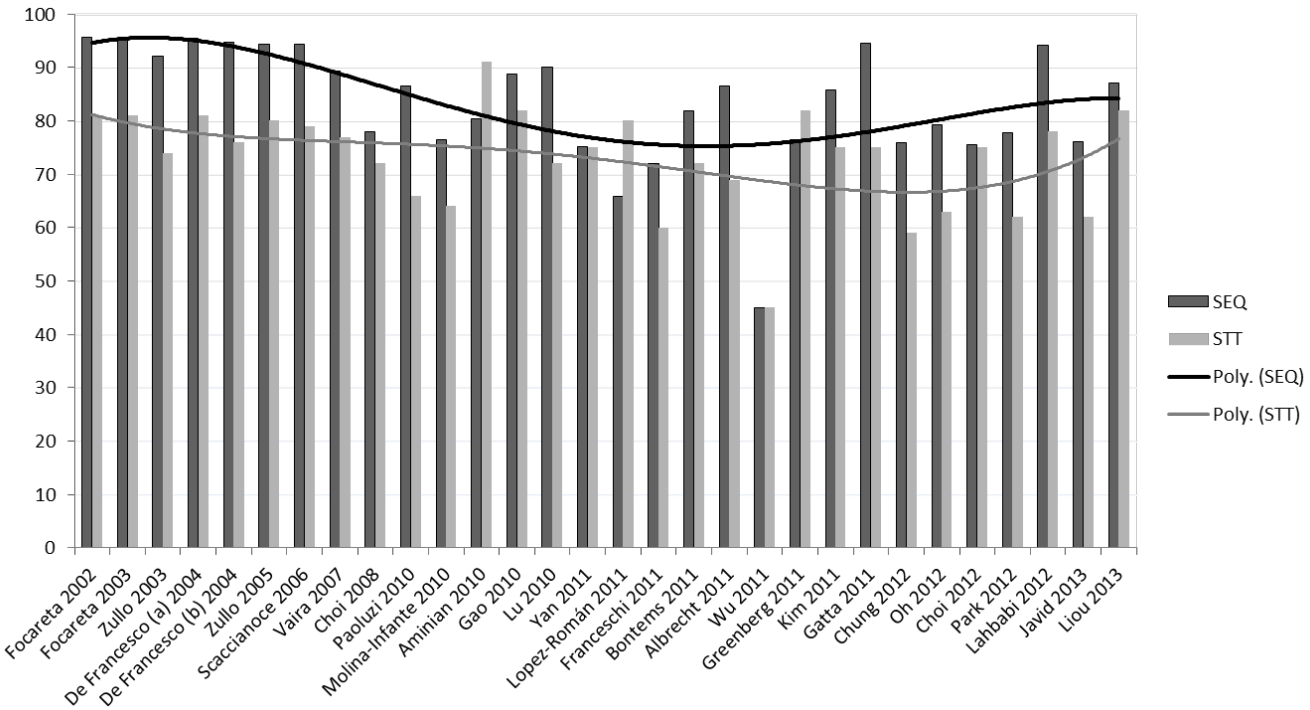
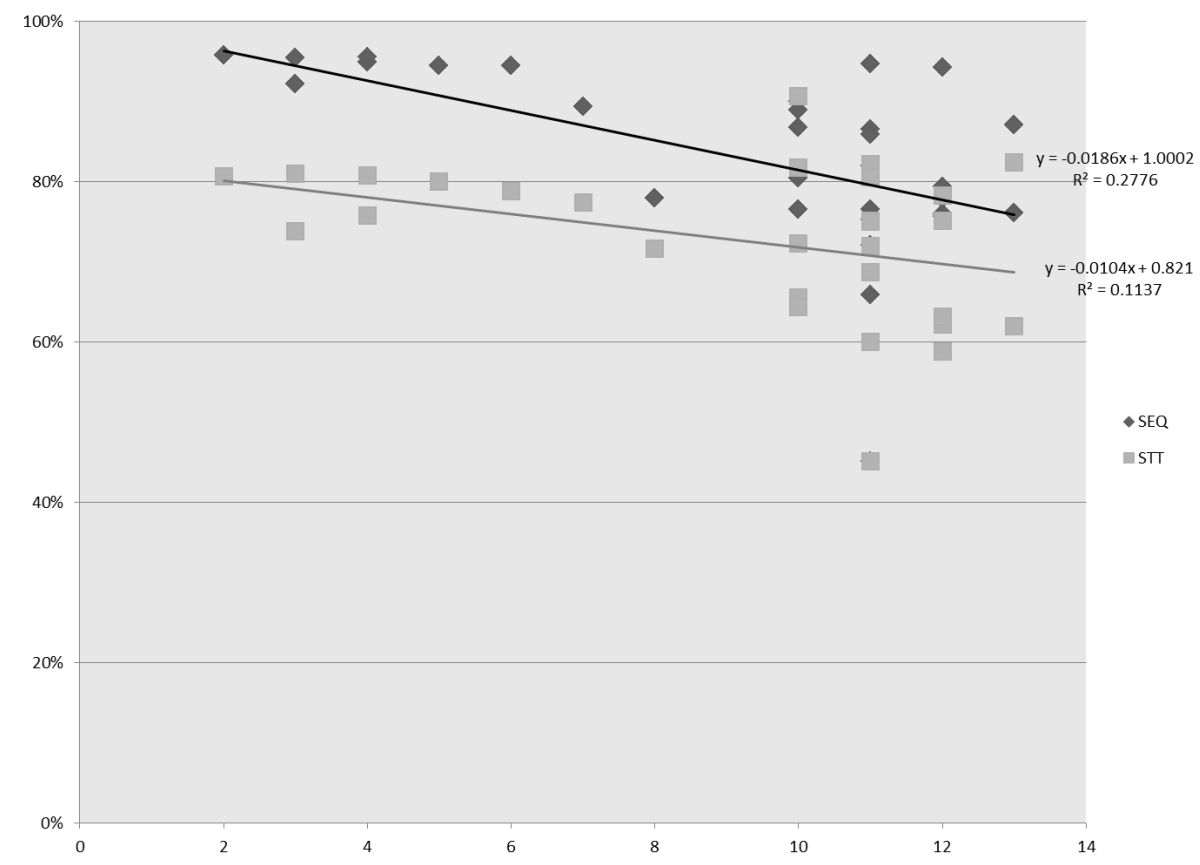


Figure 4. Ten-day SEQ and STT linear weighted regression by publication date*



*The 'x' axis represents the year of publication of the study. For instance '2' and '12' state for year 2002 and year 2012, respectively.
The 'y' axis represents the eradication rate (%) in the corresponding treatment arm.

Figure 5. ITT eradication rates (%) with 10 day SEQ through the time

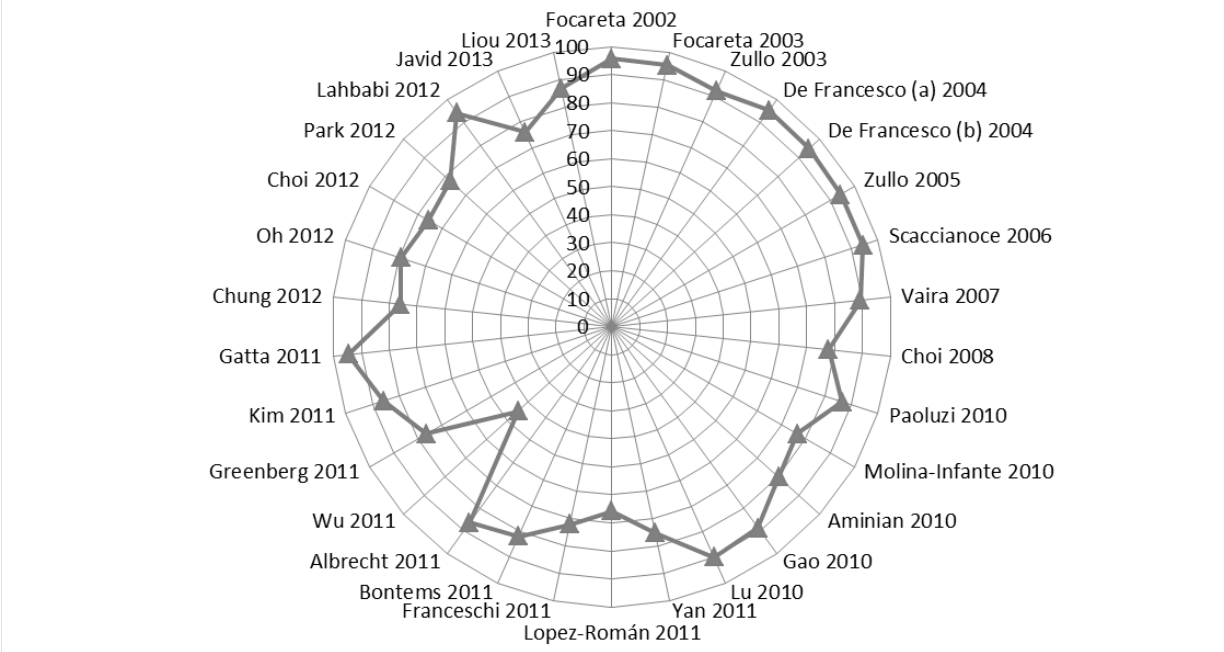


Table 2. Studies evaluating the efficacy of SEQ

First author	Country	Year of publication	Disease type	Therapy regimen	Total nº patients	ITT eradication rate (%)
Focareta	Italy	2002	PUD and NUD	O 20 mg b.i.d. + A 1 g b.i.d. 5 d O 20 mg b.i.d. + C 500 mg b.i.d. + M 500 mg b.i.d. 5 d	94	95.7
Focareta	Italy	2003	PUD and NUD	E 20 mg b.i.d. + A 1 g b.i.d. 5 d E 20 mg b.i.d. + C 500 mg b.i.d. + T 500 mg b.i.d. 5 d	174	95.4
Zullo	Italy	2003	PUD	R 20 mg b.i.d. + A 1 g b.i.d. 5 d R 20 mg b.i.d. + C 500 mg b.i.d. + T 500 mg b.i.d. 5 d	522	92.1
De Francesco (a)	Italy	2004	PUD and NUD	R 20 mg b.i.d. + A 1 g b.i.d. 5 d R 20 mg b.i.d. + C 500 mg b.i.d. + T 500 mg b.i.d. 5 d	45	95.5
De Francesco (b)	Italy	2004	PUD	R 20 mg b.i.d. + A 1 g b.i.d. 5 d R 20 mg b.i.d. + C 500 mg b.i.d. + T 500 mg b.i.d. 5 d	116	94.8
Zullo	Italy	2005	PUD	R 20 mg b.i.d. + A 1 g b.i.d. 5 d R 20 mg b.i.d. + C 500 mg b.i.d. + T 500 mg b.i.d. 5 d	89	94.3

Scaccianoce	Italy	2006	NUD	E 20 mg b.i.d. + A 1 g b.i.d. 5d E 20 mg b.i.d. + C 500 mg b.i.d. + T 500 mg b.i.d. 5 d	72	94.4
Vaira	Italy	2007	PUD	P 40 mg b.i.d. + A 1 g b.i.d. 5 d P 40 mg b.i.d.+ C 500 mg b.i.d. + T 500 mg b.i.d. 5 d	150	89.3
Choi	Korea	2008	PUD	O 20 mg b.i.d. + A 1 g b.i.d. 5 d O 20 mg b.i.d. + C 500 mg b.i.d. + M 500 mg b.i.d. 5 d	77	77.9
Paoluzi	Italy	2010	N.R	E 20 mg b.i.d. + A 1g b.i.d. 5 d E 20 mg b.i.d. + C 500 mg b.i.d. + T 500 mg b.i.d. 5 d	90	86.6
Molina-Infante	Spain	2010	PUD	O 20 mg b.i.d. + A 1 g b.i.d. 5 d O 20 mg b.i.d. + C 500 mg b.i.d. + M 500 mg b.i.d. 5 d	115	76.5
Aminian	Iran	2010	NUD	O 20 mg b.i.d. + A 1 g b.i.d. 5 d O 20 mg b.i.d. + C 500 mg b.i.d. + M 500 mg b.i.d. 5 d	107	80.3
Gao	China	2010	PUD	O 20 mg b.i.d. + A 1 g b.i.d. 5 d O 20 mg b.i.d. + C 500 mg b.i.d. + T 500 mg b.i.d. 5 d	72	88.8

Lu	China	2010	PUD and NUD	O 0.8 mg / kg / day + A 40 mg / kg / day 5 d O 0.8 mg / kg / day + A 40 mg / kg / day 5 d + C 15 mg / kg / day + T 15 mg / kg / day 5 d	40	90
Yan	China	2011	N.R	E 20 mg b.i.d. + A 1g b.i.d. 5 d E 20 mg b.i.d. + C 500 mg b.i.d. + T 500 mg b.i.d. 5 d	246	75.2
Lopez-Román	Puerto Rico	2011	N.R	O 20 mg b.i.d. + A 1 g b.i.d. 5 d O 20 mg b.i.d. + C 500 mg b.i.d. + M 500 mg b.i.d. 5 d	41	65.8
Franceschi	Italy	2011	N.R	L 15 mg b.i.d. + A 1g b.i.d. 5 d L 15 mg b.i.d. + C 500 mg b.i.d. + T 500 mg b.i.d. 5 d	50	72
Bontems	Belgium	2011	PUD	O 20 mg b.i.d. + A 1 g b.i.d. 5 d O 20 mg b.i.d. + C 500 mg b.i.d. + M 500 mg b.i.d. 5 d	83	81.9
Albrecht	Poland	2011	N.R	O 20 mg b.i.d. + A 1 g b.i.d. 5 d O 20 mg b.i.d. + C 500 mg b.i.d. + M 500 mg b.i.d. 5 d	52	86.5

Wu	China	2011	NUD and PUD	E 20 mg b.i.d. + A 1 g b.i.d. 5 d E 20 mg b.i.d. + C 500 mg b.i.d. + T 500 mg b.i.d. 5 d	102	45
Greenberg	Latin America	2011	NUD	L 30 mg b.i.d. + A 1 g b.i.d. 5 d L 30 mg b.i.d. + C 500 mg b.i.d. + M 500 mg b.i.d. 5 d	486	76.5
Kim	Korea	2011	PUD	P 40 mg b.i.d. + A 1 g b.i.d. 5 d P 40 mg b.i.d.+ C 500 mg b.i.d. + M 500 mg b.i.d. 5 d	205	85.8
Gatta	Italy	2011	NUD and PUD	E 40 mg b.i.d. + A 1 g b.i.d. 5 d E 40 mg b.i.d. + C 500 mg b.i.d. + T 500 mg b.i.d. 5 d	131	94.6
Chung	Korea	2012	PUD	L 30 mg b.i.d. + A 1 g b.i.d. 5 d L 30 mg b.i.d. + C 500 mg b.i.d. + M 500 mg b.i.d. 5 d	79	75.9
Oh	Korea	2012	PUD and NUD	R 20 mg b.i.d. + A 1 g b.i.d. 5 d R 20 mg b.i.d. + C 500 mg b.i.d. + M 500 mg b.i.d. 5 d	116	79.3
Choi	Korea	2012	PUD and NUD	R 20 mg b.i.d. + A 1 g b.i.d. 5 d R 20 mg b.i.d. + C 500 mg b.i.d. + T 500 mg b.i.d. 5 d	115	75.6

Park	Korea	2012	PUD and NUD	R 20 mg b.i.d. + A 1 g b.i.d. 5 d R 20 mg b.i.d. + C 500 mg b.i.d. + M 500 mg b.i.d. 5 d	162	77.7
Lahbabi	Morocco	2012	PUD and NUD	O 20 mg b.i.d. + A 1 g b.i.d. 5 d O 20 mg b.i.d. + C 500 mg b.i.d. + M 500 mg b.i.d. 5 d	104	94.2
Javid	India	2013	PUD	P 40 mg b.i.d. + A 1g b.i.d. 5 d P 40 mg b.i.d.+ C 500 mg b.i.d. + T 500 mg b.i.d. 5 d	138	76
Liou	China	2013	PUD	L 30 mg b.i.d + A 1 g b.i.d. 5 d L 30 mg b.i.d + C 500 mg + M 500 mg b.i.d. 5 d	300	87

ITT: intention-to-treat. RCT: randomized controlled trial. PUD: peptic ulcer disease; NUD: non-ulcer disease. PPI: proton pump inhibitor; O: omeprazole; L: lansoprazole; P: pantoprazole; R: rabeprazole; E: esomeprazole; A: amoxicillin; C: clarithromycin; M: metronidazole; T: tinidazole; d: days; b.i.d.: two times a day; t.i.d: three times a day; N.R.: not reported

In order to assess in detail the impact of different variables in the eradication rate among included trials, several subgroup analyses were performed (section 4.2.2).

4.2.2. Effects of different variables on the efficacy of sequential therapy (SEQ) vs. standard triple therapy (STT)

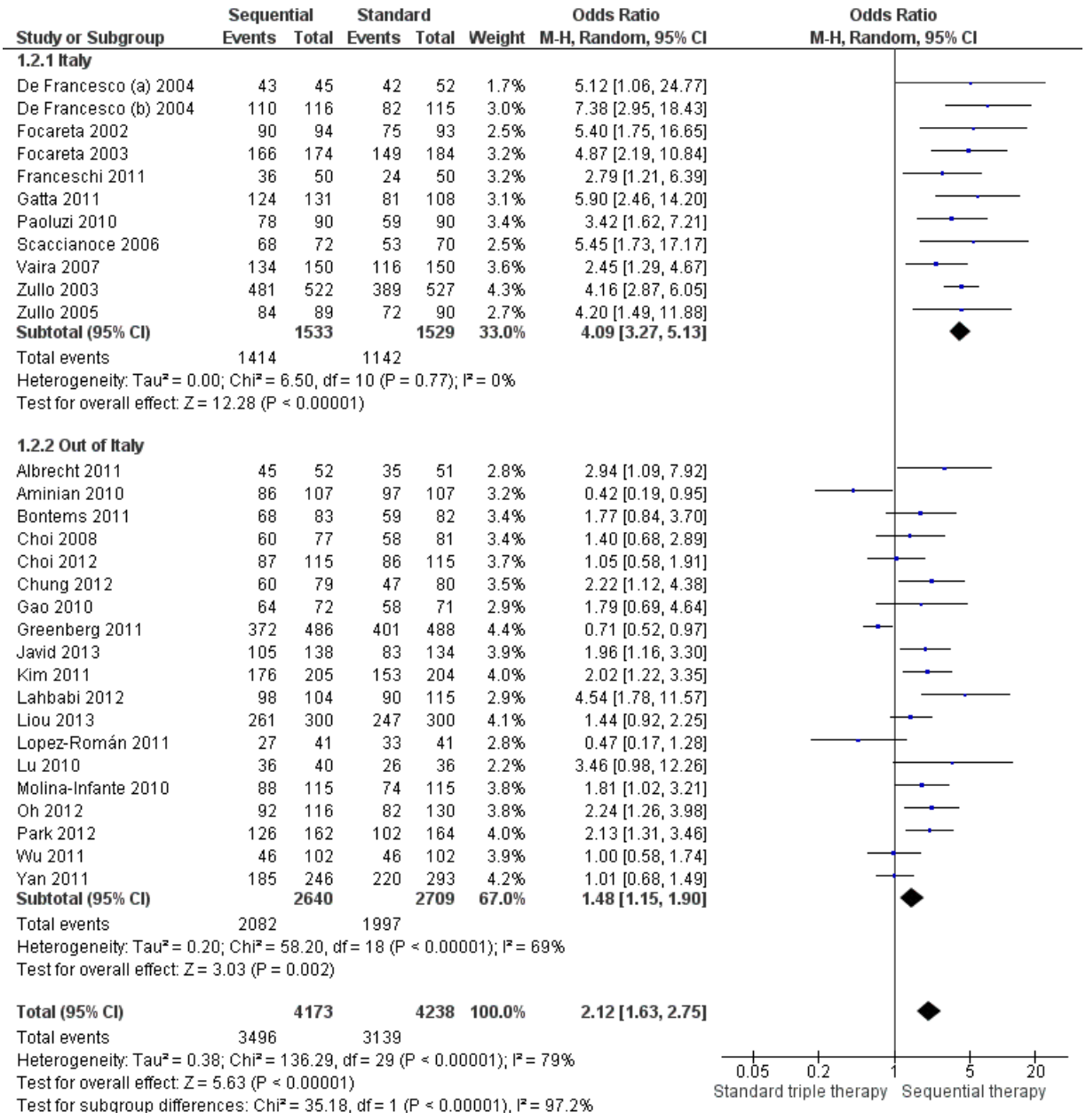
As shown in the headings below, the efficacy of the SEQ might have been influenced by several factors.

4.2.2.1. Geographic region

Around one third (37%) of the studies included in current meta-analysis were performed in Italy. Although sequential regimen was superior to standard triple regimen in both subgroup analyses (Figure 6), a greater effect size (OR 4.09 vs. 1.48) as well as a clear tendency towards higher eradication rates with SEQ was confirmed among the Italian publications (92% vs. 79%).

The NNT among the Italian studies was 6 whereas within non-Italian studies it was calculated as 15.

Figure 6. Forest plot of comparison: SEQ vs. STT, outcome: Geographic region



4.2.2.2. Publication date

Following the analysis of the eradication rate trend mentioned in the heading above (4.2.1 Overall *H. pylori* eradication rate), the year 2008 was chosen as the threshold to perform a sub-analysis accounting for the timing of the publication of the included studies.

As stated, the overall eradication rate following SEQ showed a tendency into a lower efficacy from year 2008 (Figure 3 and Figure 5). Additionally, the eradication rates before year 2008 appeared constant (or similar) between studies but after year 2008 cure rates were shown irregular or even

puzzled through the time as represented by the various plots around the trend-line between 2008 and 2012 (Figure 4).

The forest plot (Figure 7) also presented the aforementioned differences in the eradication rates of the studies performed before and after year 2008.

Before 2008, studies reported higher eradication rates and the overall effect size of the estimate was almost 2.5 times greater than among studies published after 2008 (OR 3.63 vs. 1.64). The NNT within the subgroup of studies 'before 2008' and 'after 2008' was 7 and 12 respectively.

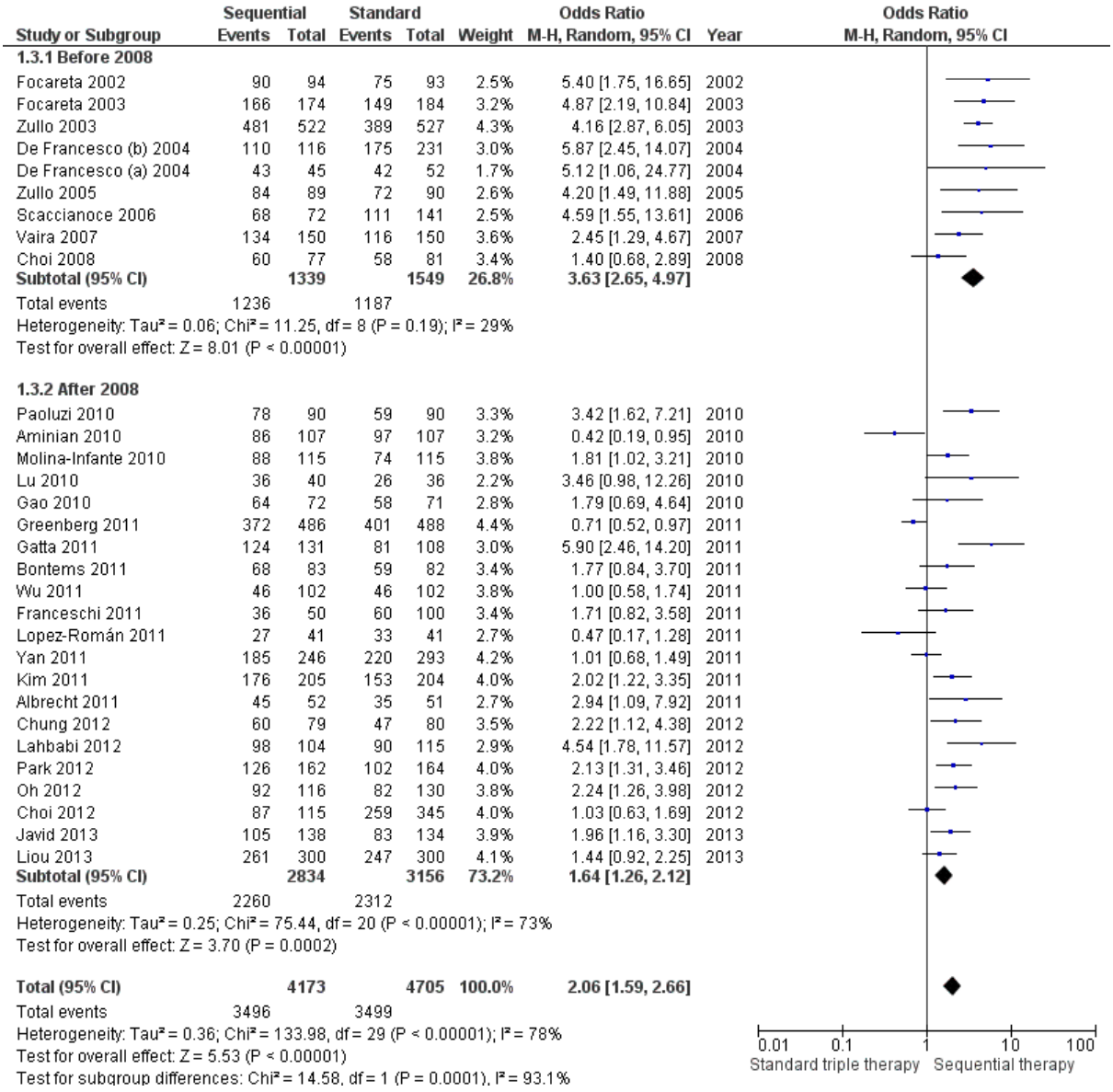
Moreover, all the studies published before the chosen year-cut-off point were performed in Italy (but one published in Korea)[25] and heterogeneity was reported moderate (I^2= 29%) whereas in studies published after 2008 it was reported higher (I^2=73%). It was decided to include the Korean study within this 'before 2008' group as it seemed more coherent given the chronological sequence of published articles.

Further sensitivity analysis, first including all studies and second excluding the Korean trial showed very similar effect size (OR 4.11 vs. 3.63) for this subgroup and no heterogeneity was recorded (I^2= 0). This analysis indicated certain robustness in the findings towards the decisions made.

Differences between SEQ and STT were significant in all Italian studies but in the Korean study mentioned (Choi et al, 2008) in which significant differences between therapies were not reported. Additionally, two Italian studies[54, 55] reported a significantly larger effect size of SEQ over STT in the 'after 2008' subgroup.

In order to prove findings were not result of arbitrary decisions, the meta-analysis was doubled and primary analysis was substituted by pooling only the data of the studies published 'after 2008'. The effect size indeed was lower (OR 1.55 vs. 2.02) however the clinical benefit with SEQ over STT could still be verified.

Figure 7. Forest plot of comparison: SEQ vs. STT, outcome: Publication date

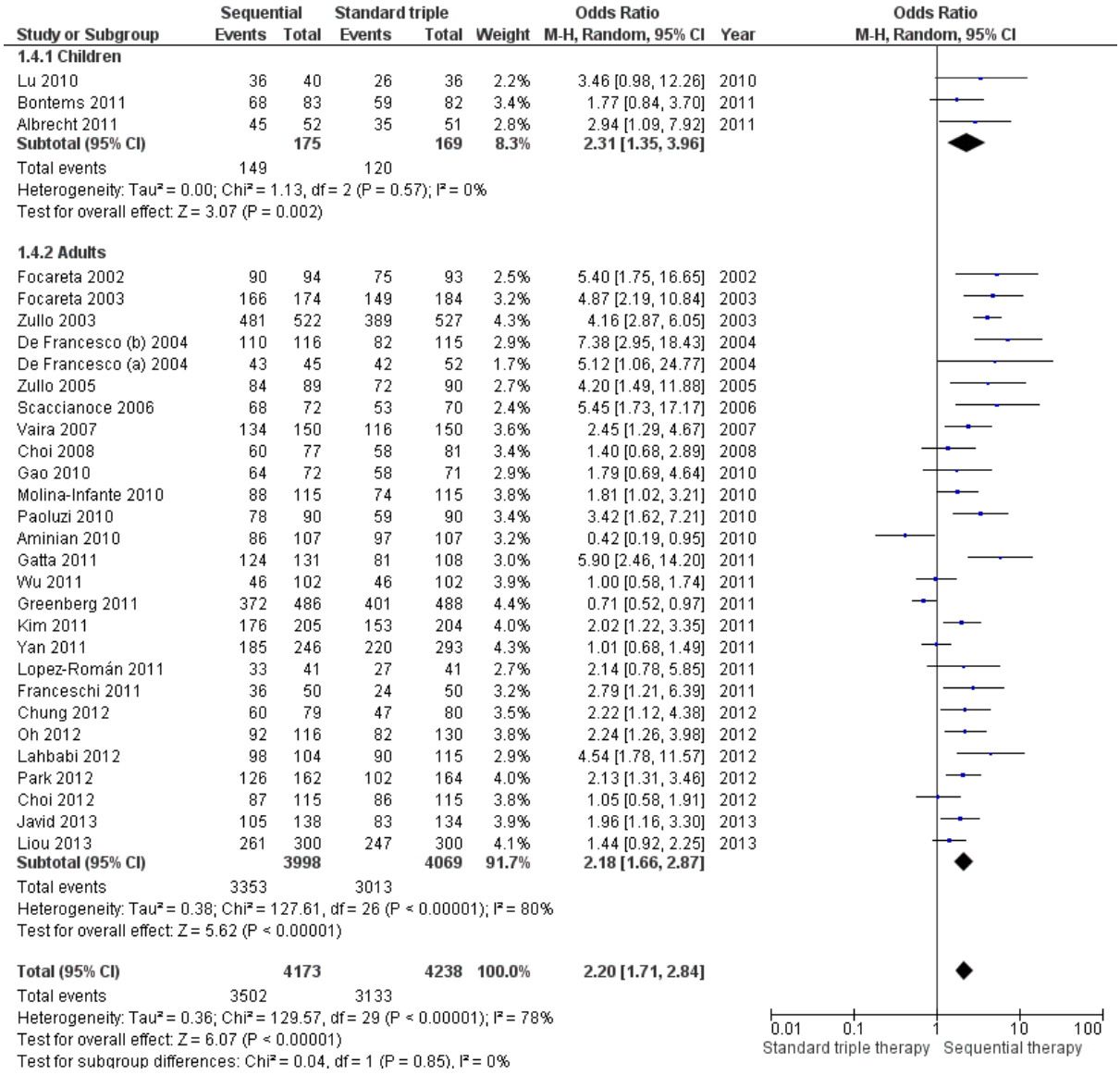


4.2.2.3. Age of the population

All included studies but 3[69, 72, 74] assessed adult patients and studies assessing children were first published in 2010 onwards.

The pooled OR for eradication of *H. pylori* with SEQ compared to STT in children was reported slightly higher than in adults (OR 2.31 vs. 2.18; Figure 8). The NNT was 8 in children and 9 in adults.

Figure 8. Forest plot of comparison: SEQ vs. STT, outcome: Age of the population

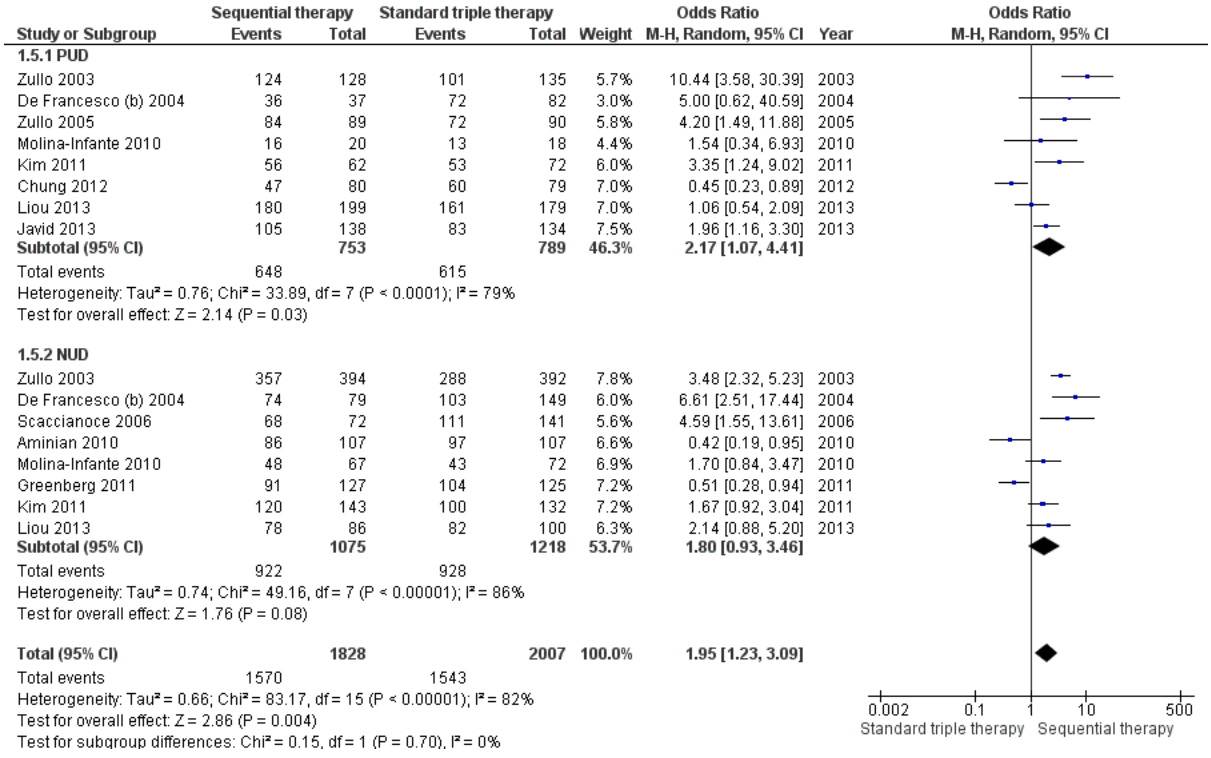


4.2.2.4. Type of underlying disease: non-ulcer disease (NUD) vs. peptic ulcer disease (PUD)

Eleven studies reported the baseline medical condition of patients. A total of 1,542 and 2,293 patients were assessed in the PUD and NUD subgroups, respectively.

Data of the present meta-analysis suggest that eradication rates following SEQ in both ulcer patients and non-ulcer patients were equal (86%) but differences between SEQ and STT were significant only among PUD patients (OR= 2.17; [95%CI= 1.07-4.41]; p<0.001) in which SEQ could demonstrate to be more effective (Figure 9). The NNT among PUD and NUD patients was 7 and 11 respectively.

Figure 9. Forest plot of comparison: SEQ vs. STT, outcome: Medical condition



4.2.2.5. Length of the standard triple therapy (STT)

Sixteen, 11 and 5 studies assessed respectively 7-day, 10-day and 14-day STT vs. 10-day SEQ. Eradication rates and ORs are summarized in Table 3.

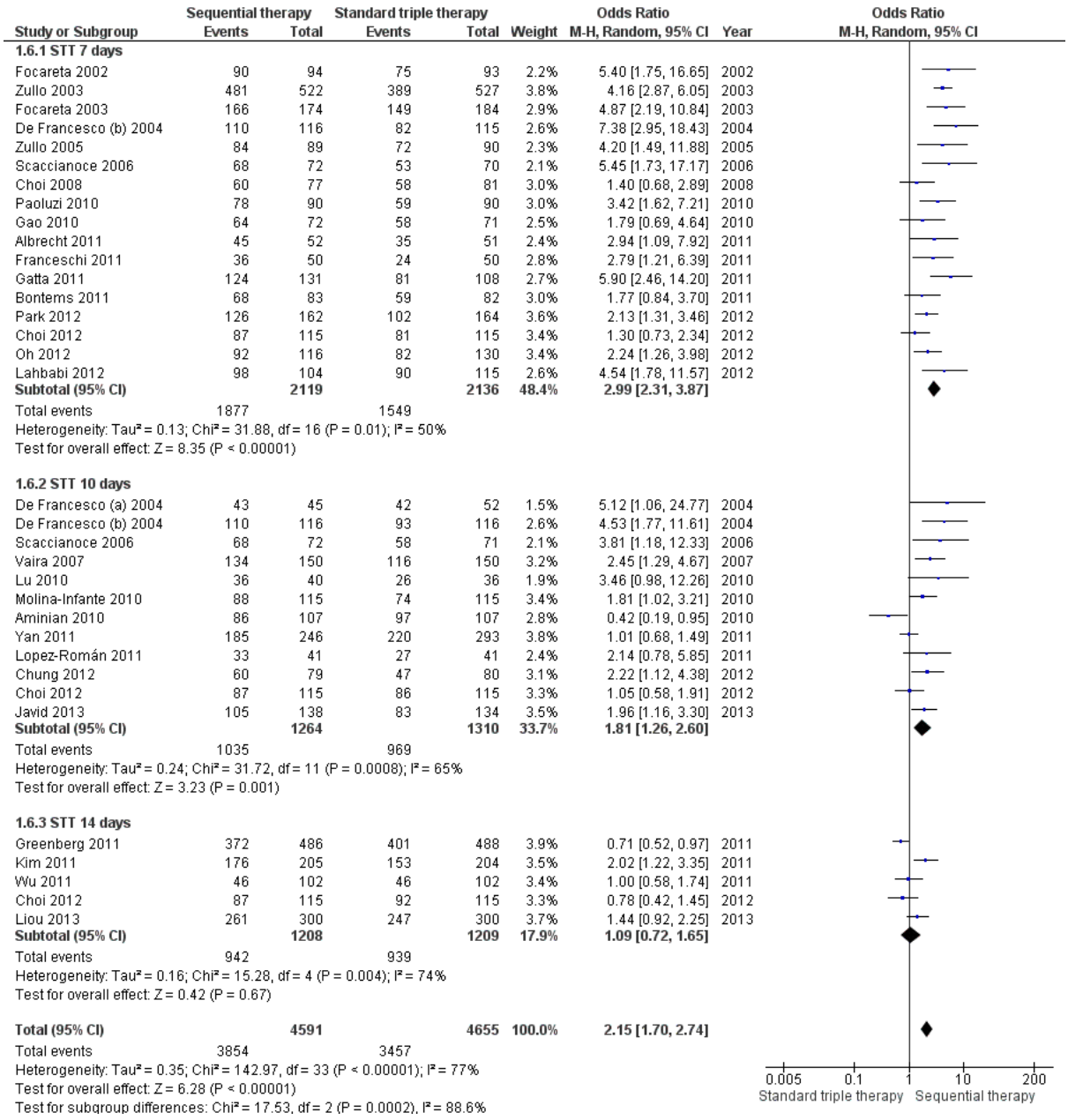
Table 3. Efficacy of 7-day, 10-day and 14-day STT vs. 10-day SEQ

	7-d STT vs. 10-d SEQ	10-d STT vs. 10-d SEQ	14-d STT vs. 10-d SEQ
ITT eradication rates (%)	72.5 vs. 88.5	73.9 vs. 81.8	77.9 vs. 77.6
OR [95%CI]	2.93 [2.31, 3.87]*	1.81 [1.26, 2.60]*	1.09 [0.72, 1.75], NS

STT: standard triple therapy; SEQ: sequential therapy; OR: odds ratio.
*Differences were significant between treatment groups. N.S: non-significant differences

SEQ was significantly better than 7-day and 10-day standard triple therapies but differences between 14-day STT and 10-day SEQ were not observed (p=0.67; Figure 10). The NNT within both the STT 7-day and STT – 10 days subgroup was 7 and 11 respectively.

Figure 10. Forest plot of comparison: SEQ vs. STT, outcome: STT length



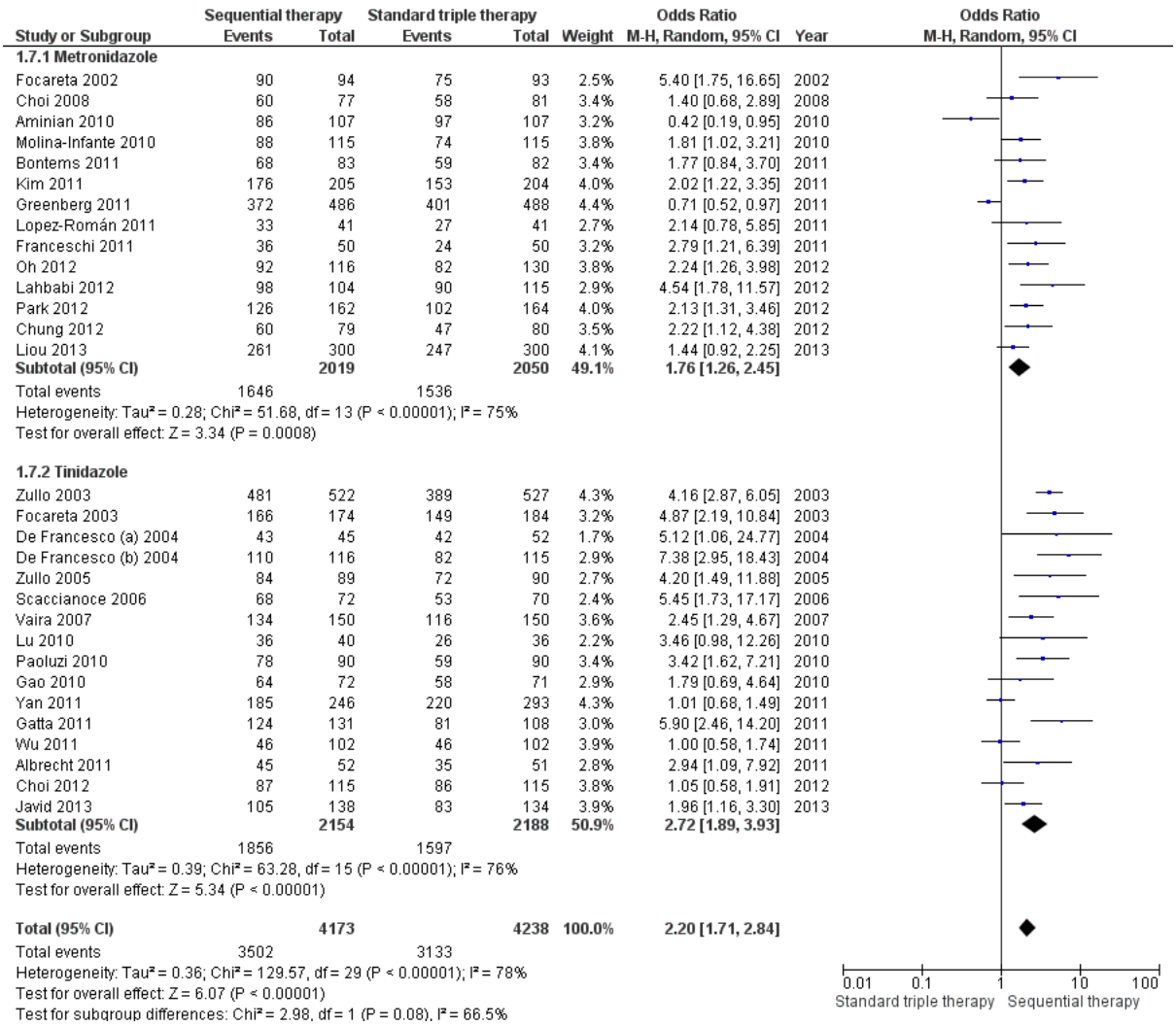
4.2.2.6. Type of nitroimidazole

Fourteen and 16 studies used metronidazole and tinidazole respectively in patients treated with SEQ.

The subgroup analysis showed that SEQ was significantly better than STT when patients were treated with either metronidazole or tinidazole. However, the effect size when patients were given tinidazole was higher than in those treated with metronidazole (OR 2.72 vs. 1.76), suggesting a

benefit of this drug with SEQ (Figure 11). The NNT among both the tinidazole and metronidazole subgroup was 7 and 11 respectively.

Figure 11. Forest plot of comparison: SEQ vs. STT, outcome: Nitroimidazole type



4.2.2.7. Proton-pump inhibitor (PPI) type and dosing

Both STT and SEQ used different PPIs (omeprazole, lansoprazole, pantoprazole, rabeprazole, or esomeprazole) as well as different PPI doses among the included studies.

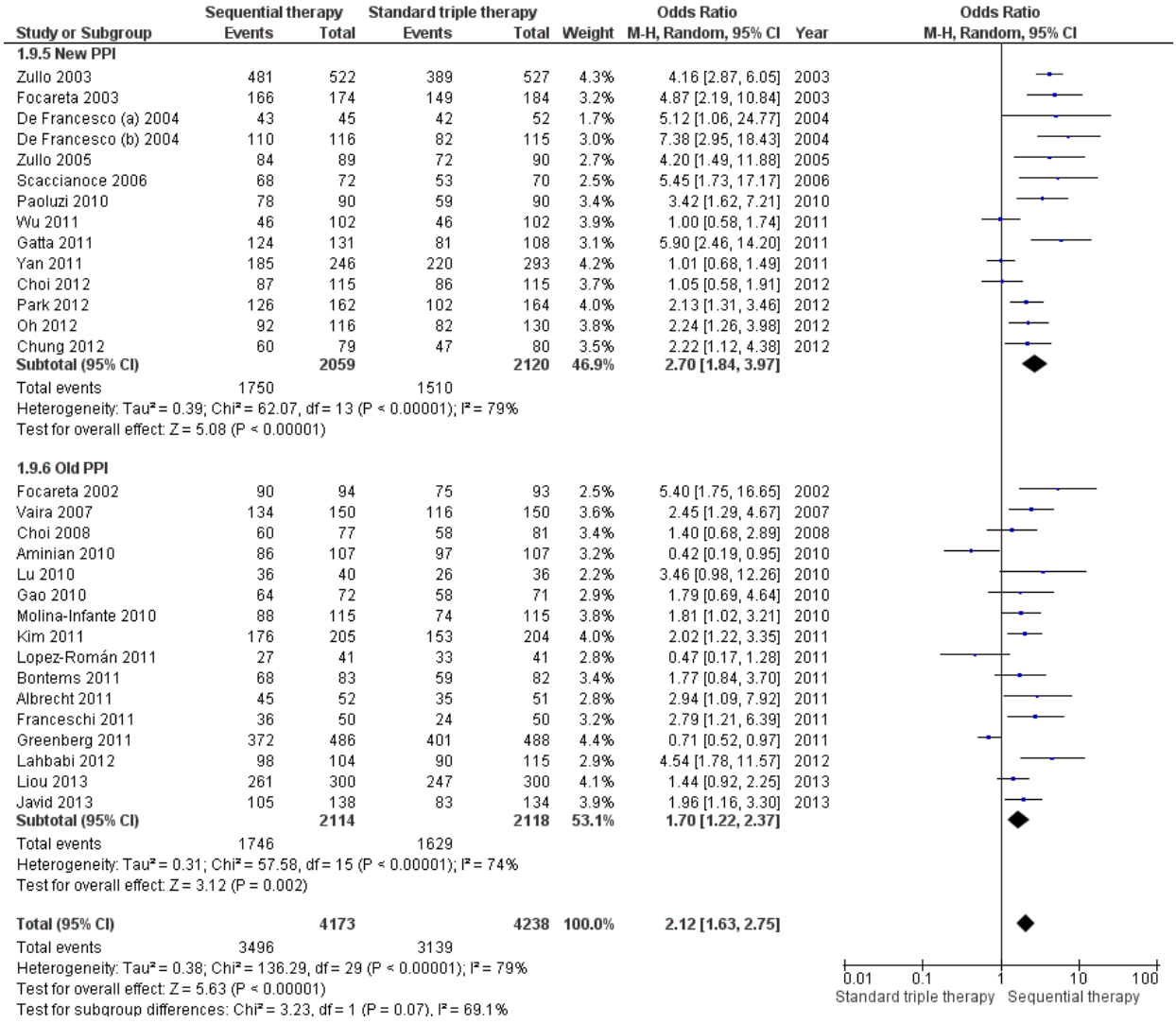
A subgroup analysis was performed to compare the efficacy of adjuvant medication within both treatment regimens. Studies in the 'new generation PPIs' group used either esomeprazole or rabeprazole and studies in the 'old / first generation PPIs' used omeprazole, lansoprazole or pantoprazole. Fourteen studies (46%) assessed the new generation of PPIs and the rest evaluated first/old generation PPIs.

The data presented SEQ as more effective than STT using the new PPI generation (esomeprazole or rabeprazole) than when patients were given a first PPI generation (omeprazole or pantoprazole) (OR 2.70 vs. 1.70; Figure 12).

The number needed to treat within studies using the new generation PPIs was 7.

Regarding dosage, almost all studies used standard doses (20 mg twice daily) for omeprazole, rabeprazole or esomeprazole as well as for pantoprazole (40 mg twice daily) and lansoprazole (30 mg twice daily). One Italian study[54] used esomeprazole at double-doses (40 mg twice daily) in an adult population. Another Italian study[53] used low-dose lansoprazole (15 mg twice daily) in both treatment arms.

Figure 12. Forest plot of comparison: SEQ vs. STT, outcome: PPI type



4.2.2.8. Antibiotic resistance

Clarithromycin, nitroimidazole and the dual clarithromycin and nitroimidazole resistances did not result in significant differences in any of the subgroup analyses between both regimens' eradication rates (Figure 13).

Despite antibiotic resistances, when assessing the total of patients for whom antibiotic resistances had been assessed (273 in the SEQ arm and 268 in the STT arm), SEQ was significantly more beneficial than STT (OR= 2.42; [95%CI 1.05-5.58]; p=0.04).

Moreover, patients with clarithromycin-resistant strains showed a greater benefit of SEQ over STT than in the remaining subgroup analyses assessing antibiotic resistance. Additionally, the effect estimate within the clarithromycin resistance subgroup analysis was also reported greater if compared to the effect estimate of the overall eradication rate analysis (OR 4.20 vs. 2.12), meaning differences between treatment arms were even greater among those patients with primary resistances.

On the other hand, the subgroup analysis evaluating patients with nitroimidazole-resistant strains presented no differences between treatments but patients could benefit more from SEQ (87%) than with STT (82%) and eradication rates among patients with secondary resistances were higher in both treatment arms than in those patients with primary resistances. However, the effect size was reported lower than in those with clarithromycin-resistant strains (OR 1.74 vs. 4.20).

Patients with dual clarithromycin and nitroimidazole resistance did not report significant differences between SEQ and STT, although eradication rates with SEQ (80%) were greater than with STT (67%).

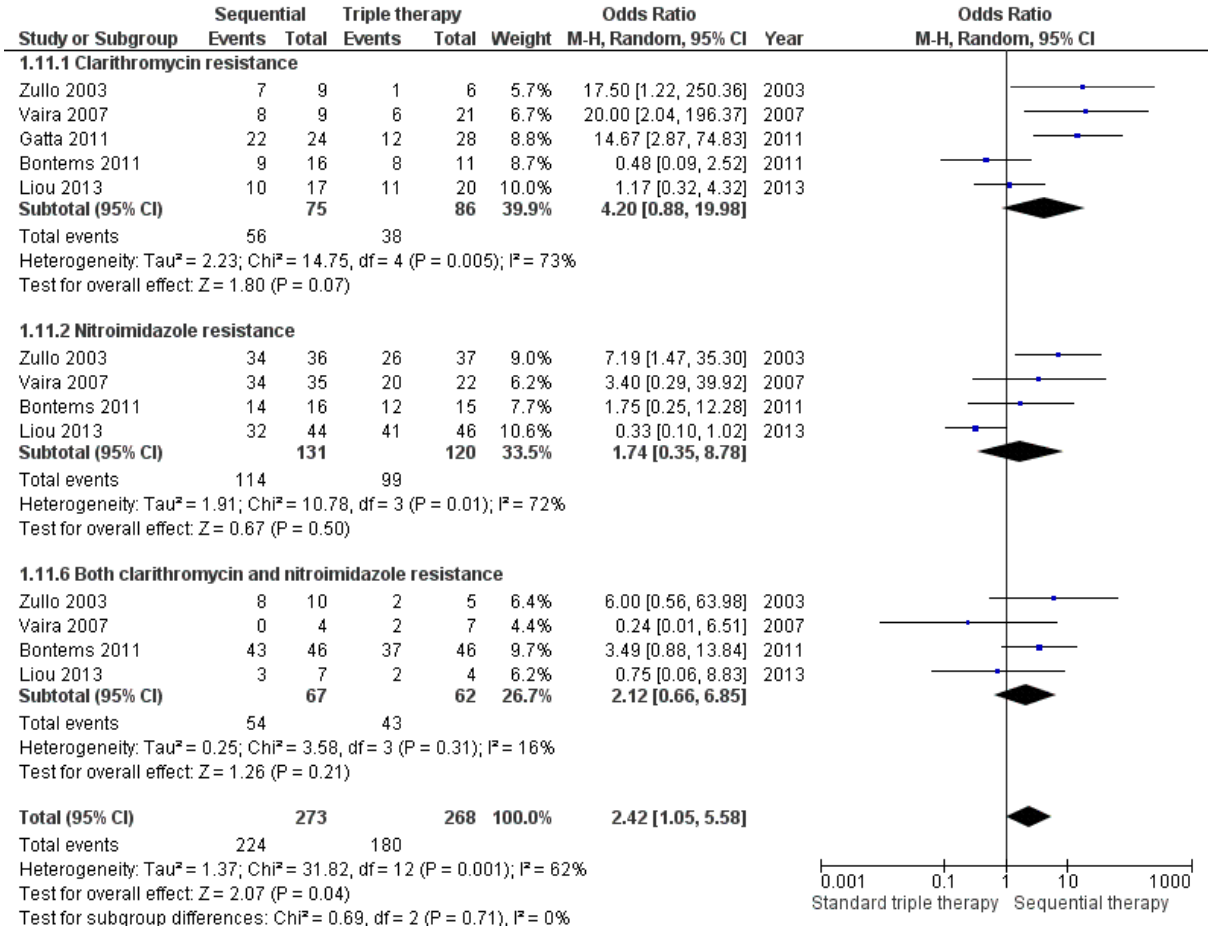
Figures regarding antibiotic susceptibility were not available among most of the studies reporting resistance; and therefore data could not be pooled into the corresponding forest plot. Only two studies,[57, 68] reported antibiotic susceptibility for clarithromycin, nitroimidazole and the combination of both (Table 4).

Table 4. Reported antibiotic susceptibilities by treatment arm

		clarithromycin-S		**nitroimidazole-S**		**clarithromycin and nitroimidazole-S**	
		SEQ	STT	SEQ	STT	SEQ	STT
Vaira	2007	108 / 114	86 / 91	83 / 88	72 / 90	N.R	N.R
Liou	2013	152 / 166	137 / 151	130 / 139	107 / 125	123 / 129	98 / 109

S: susceptibility. N.R. not reported

Figure 13. Forest plot of comparison: SEQ vs. STT, outcome: Antibiotic resistance



4.2.2.9. Compliance and tolerance

Twenty-two (73%) studies described common adverse events (AEs) such as abdominal pain, diarrhoea, nausea, glossitis and vomiting and their incidence by treatment arms. None of the studies reported any serious AE (Table 5).

Within the SEQ arm the incidence of AEs ranged from 7% in the study by Zullo in 2003[59] to 54% in the study by Paoluzi in 2010,[55] whereas within the STT the incidence ranged from 2% in the study by Aminian in 2010[70] to 55% in the study by Liou in 2013.[68]

In the ITT analysis, the overall adverse event rate (Figure 14) showed no significant differences between SEQ and STT (18% in both treatment arms; OR= 0.96; [95%CI=0.84-1.10]; p=0.56). Results were homogeneous (I^2=11%), therefore a fixed effect model was used as appropriate. None of the studies was able to demonstrate differences between therapies' side effects.

Similar compliance rates were reported among the 22 studies but for the study published by Park et al in 2012[64] rates were reported lower: 72% and 58% with SEQ and STT respectively. With the exception of the aforementioned study, compliance rates ranged as follows: from 85% in the study by Aminian et al in 2010[70] to 97% in the study by Kim et al in 2011[62] with SEQ and from 81% in the study by Liou et al in 2013[68] to 97% in the study by Kim et al in 2011[62] with STT.

Overall, AEs required the interruption of therapy in 18 patients out of a total of 596 (3%) experiencing AEs in the SEQ arm and in 35 out of 664 (5%) in the STT arm (Table 5).

Figure 14. Forest plot of comparison: SEQ vs. STT, outcome: adverse events.

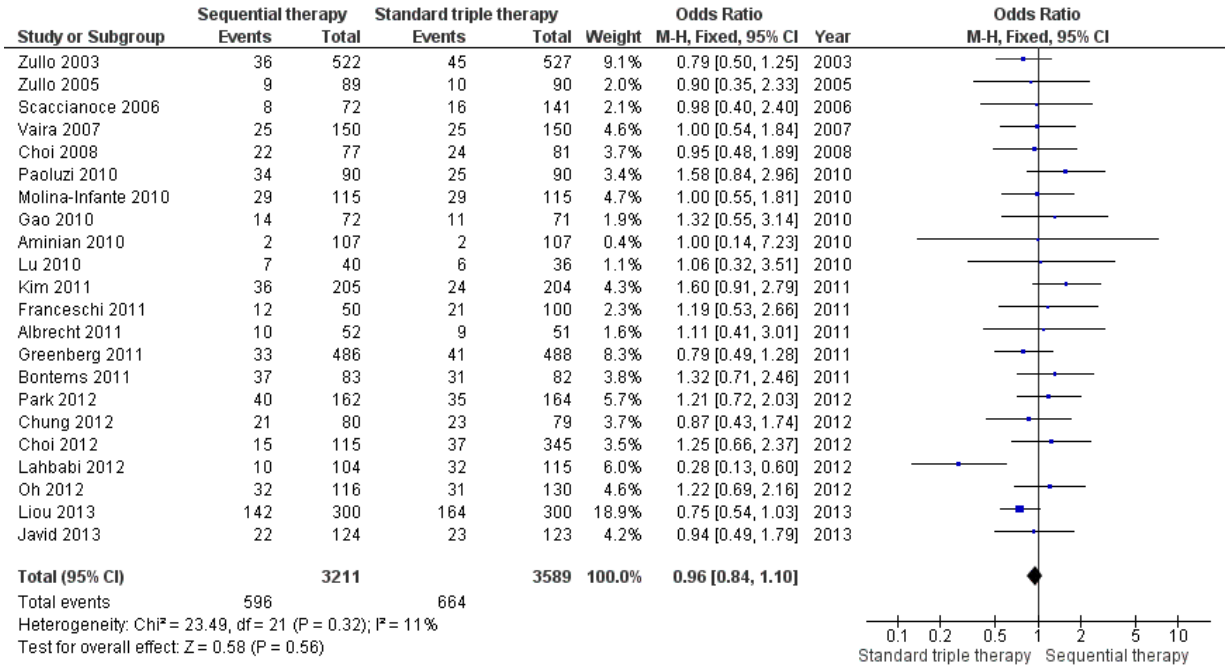


Table 5. Compliance, AEs and withdrawals (due to AEs) within both treatment arms

		Compliance rate (%)*				AEs rate (%)				Withdrawals rate (%) due to AEs			
First author	**Year**	**10d- SEQ**	**7d- STT**	**10d- STT**	**14d- STT**	**10d- SEQ**	**7d- STT**	**10d- STT**	**14d- STT**	**10d- SEQ**	**7d- STT**	**10d- STT**	**14d- STT**
Zullo	2003	456 / 522 (90)	471 / 527 (93)			36 / 522 (7)	45 / 527 (9)			0 / 522 (0)	1 / 527 (0.2)		
Zullo	2005	> 95%	> 95%			9 / 89 (10.1)	10 / 90 (11.1)			1 / 89 (1.1)	2 / 90 (2.2)		
Scaccianoce	2006	> 95%	> 95%	> 95 %		8 / 72 (11.1)	7 / 70 (10)	9 / 71 (12.7)		2 / 72 (2.7)	2 / 70 (2.8)	2 / 71 (2.8)	
Vaira	2007	135 / 150 (90)**		135 / 150 (90)**		25 / 150 (16.6)**		25 / 150 (16.6)**		0 / 150 (0)**		1 / 150 (0.6)*	
Choi	2008	N.R	N.R			22 / 77 (28.6)	24 / 81 (29.6)			N.R	N.R		
Paoluzi	2010	> 90%	> 90 %			34 / 90 (54)	25 / 90 (42)			3 / 90 (3.3)	2 / 90 (2.2)		
Molina-Infante	2010	111 / 115 (96.5)	114 / 115 (99.1)			29 / 115 (25)	29 / 115 (25)			1 /115 (0.8)	1 / 115 (0.8)		
Gao	2010	> 95%	> 95%			14 / 72 (19.4)	11 / 71 (15.5)			0 / 72 (0)	0 / 71 (0)		
Aminian	2010	> 85%		> 85%		2 / 107 (1.9)		2 / 107 (1.9)		N.R		N.R	
Lu	2010	N.R		N.R		7 / 40 (17.5)		6 / 36 (16.6)		N.R		N.R	
Albrecht	2011	> 95%	> 95%			10 / 52 (19.2)	9 / 51 (17.6)			0 / 52	0 / 51		
Franceschi	2011	N.R	N.R			12 / 50 (24)	21 / 100 (21)			N.R	N.R		
Kim	2011	199 / 205 (97)**			199 /204 (97.5)**	36 / 205 (17.5)**			24 / 204 (11.7)**	0 / 205 (0)			0 / 204 (0)

Greenberg	2011	427 / 486 (87.8)**			437 / 488 (89.5)**	33 / 486 (6.7)**			41 / 488 (8.4)**	N.R			N.R
Bontems	2011	N.R	N.R			37 / 83 (44)	31 / 82 (37.8)			N.R	N.R		
Choi	2012	> 95%	> 95%	> 95%	> 95%	15 / 115 (13)	11 / 115 (9.5)	14 / 115 (12.1)	12 / 115 (10.4)	N.R	N.R	N.R	N.R
Oh	2012	> 90%	> 90%			32 / 116 (27.5)	31 / 130 (23.8)			0 / 116 (0)	0 / 130 (0)		
Park	2012	116 / 162 (71.6)	95 / 164 (57.9)			40 / 162 (24.7)	35 / 164 (21.3)			2 / 162 (1.2)	2 / 164 (1.2)		
Chung	2012	76 / 79 (96.2)		77 / 80 (96.2)		21 / 80 (26.3)		23 / 79 (29.1)		3 / 79 (3.8)		3 / 80 (3.7)	
Lahbabi	2012	98 / 104 (94.2)	106 / 115 (92.2)			10 / 104 (9.6)	32 / 115 (27.8)			0/104	5 / 115 (4.3)		
Javid	2013	121 / 124 (97.6)		121 / 123 (98.4)		22 / 124 (17.7)		23 / 123 (18.7)		0/124		1 / 123 (0.8)	
Liou	2013	258 /300 (95)		243 / 300 (81)		142 / 300 (47.3)		164 / 300 (54.6)		6 /300 (2)		13 /301 (4.3)	

Striped pattern cells show when treatment arm was not in the trial.
N.R.: Not reported in the publication
* as defined by authors (usually good if >90% or >95% of drug intake)
* Kim, 2011 reported PP compliance rates, SEQ 184 / 190 (96.8) and 14d-STT: 175 / 180 (97.2). AEs were also as per protocol rates, SEQ: 36 / 190 (18.9) and 14d- STT: 24 / 180 (13.3)
** Vaira, 2007 reported PP compliance rates, SEQ: 135 / 143 (94) and STT: 135 / 146 (93). AEs were also PP rates, SEQ: 25 / 143 (17.5) and STT: 25 / 146 (17.1)
** Greenberg, 2011 reported PP compliance rates, SEQ: 427 / 475 (97) and STT: 437 / 470 (93). AEs were also reported PP rates, SEQ: 33 / 470 (7) and STT: 41 / 475 (9)
** Park, 2012 reported PP compliance rates, SEQ: 116 / 132 (87.9) and STT: 95 / 125 (76). AEs were also reported PP rates, SEQ: 40 / 143 (28) and STT: 35 / 137 (25.5)
** Liou, 2013 reported PP compliance rates, SEQ: 258 /285 (91) and STT: 243 / 278 (87). AEs were also reported PP rates, SEQ: 142 / 294 (48) and STT: 164 / 198 (55) and PP withdrawals rates (due to AEs) as, SEQ: 6 /295 (2) and STT: 13 /297 (4)

4.3. Risk of bias in included studies

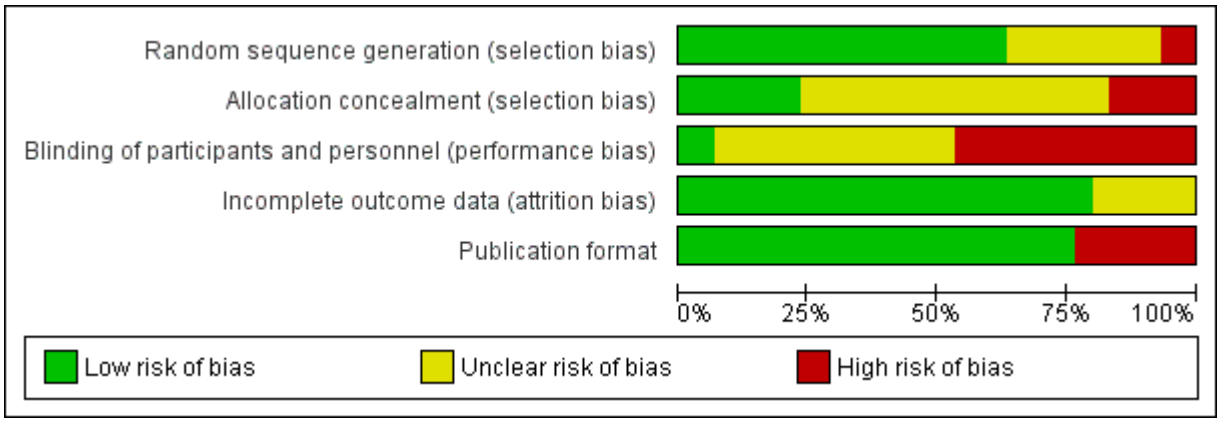
In the overall comparison 'eradication rate of SEQ vs. STT', two[57, 72] studies out of 30 were categorised as 'low risk of bias' in all 4 items of the checklist assessing the quality of the methodology (Figure 16).

Two studies,[69, 74] were categorised as 'high risk' in the items relating to randomisation, allocation and blinding. Two other studies[55, 64] were likewise flagged as having poor allocation concealment and blinding.

Lack of comprehensive reporting of outcomes as well as scarcity in the report of the information related to the assessed quality-items within the aforementioned studies, made both selection and performance biases a threat to the validity of the review (Figure 15 and Figure 16).

However, regardless of the potential biases, the subgroup analyses confirmed a significant gain in the ITT eradication rate with 10-day SEQ compared to STT (4.3.1 Allocation and 4.3.2 Blinding).

Figure 15. Risk of bias: review authors' judgements about each risk of bias item presented as percentages across all included studies



Most of the studies (63%) were reported as 'truly randomized' and were unlikely to have been subject to selection bias due to a lack of randomization of the sequence generation.

Performance bias due to lack of poor blinding of study participants and personnel was a priori the quality-item that was more likely to influence the review's outcome as only per the quality assessment over 50% of studies were flagged as 'high risk'. However, the importance of this finding in the context of *H. pylori* eradication is low, as will be analysed in the Discussion section.

Forest plot comparing the different subgroups in regards to the process of randomization across included studies is presented in Figure 17.

Both Mantel-Haenszel odds ratios (ORs) in either 'truly randomized' (OR=2.21; [95%CI=1.59-3.07]; p<0.001) or 'pseudo-randomized' (OR=2.20; [95%CI=1.45-3.33]; p<0.001) subgroups showed that patients could benefit more from the SEQ versus the STT.

Figure 16. Risk of bias summary: review authors' judgements about each risk of bias item for each included study

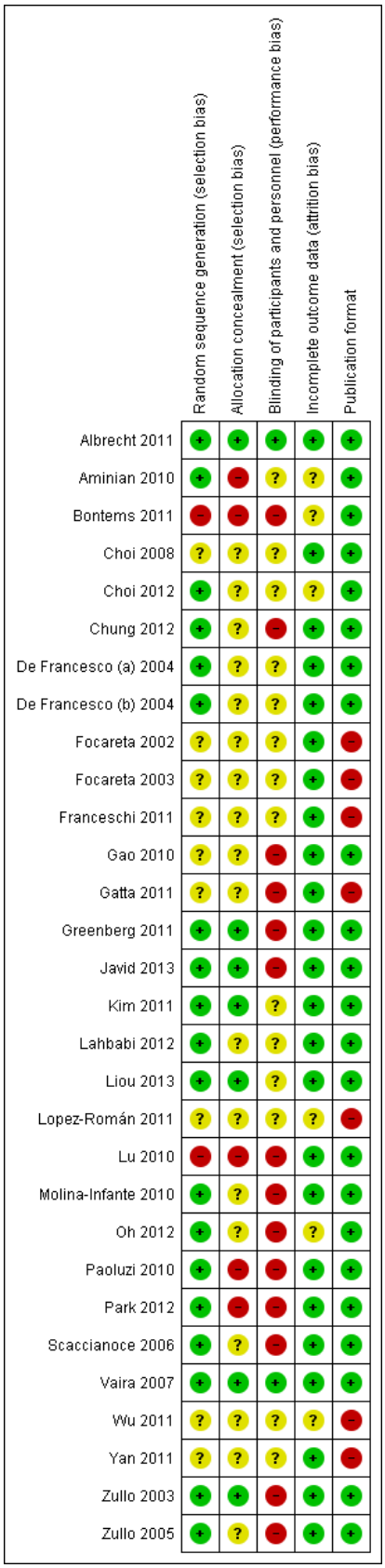
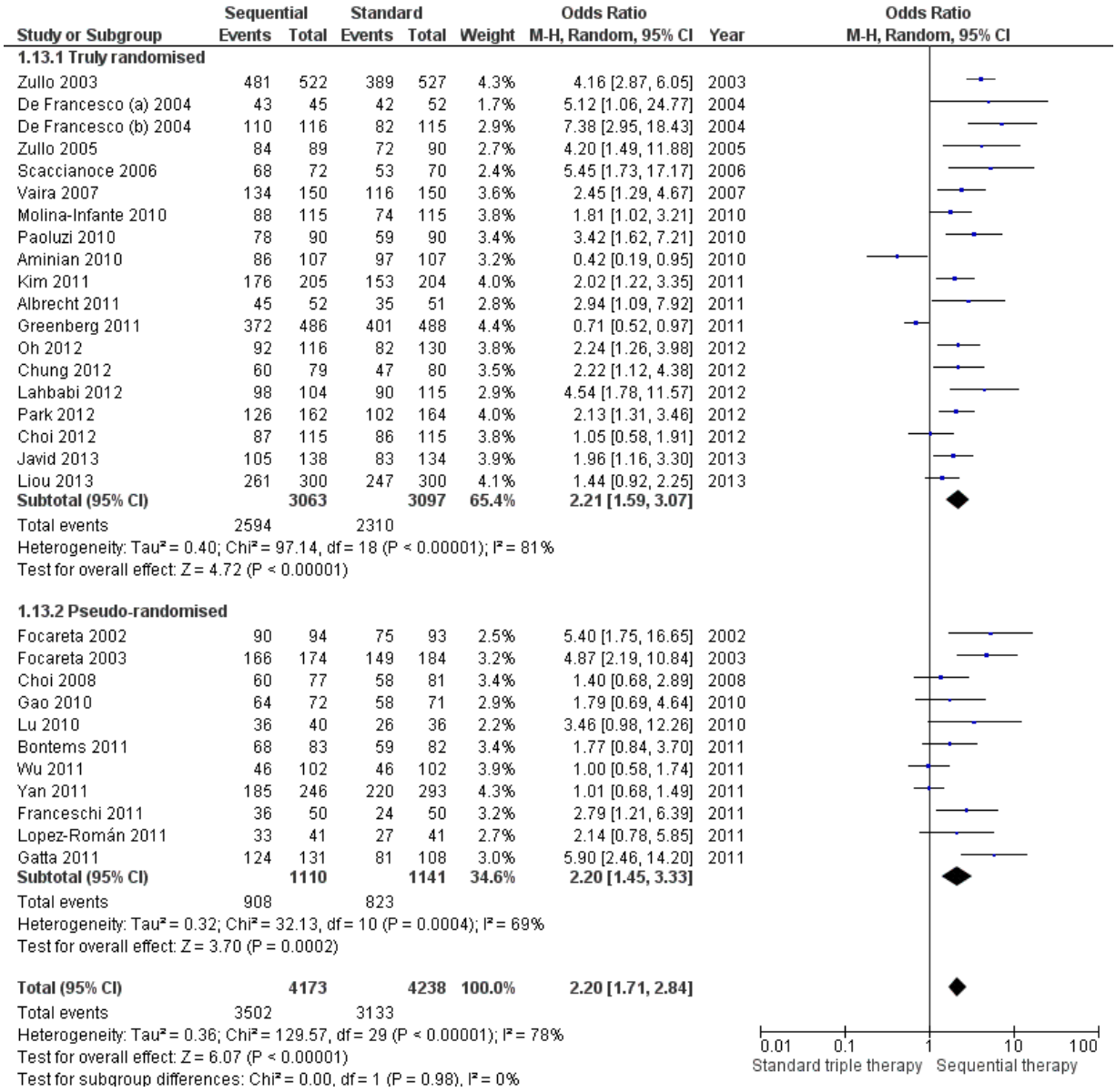


Figure 17. Forest plot of comparison: SEQ vs. STT, outcome: Randomization



4.3.1. Allocation

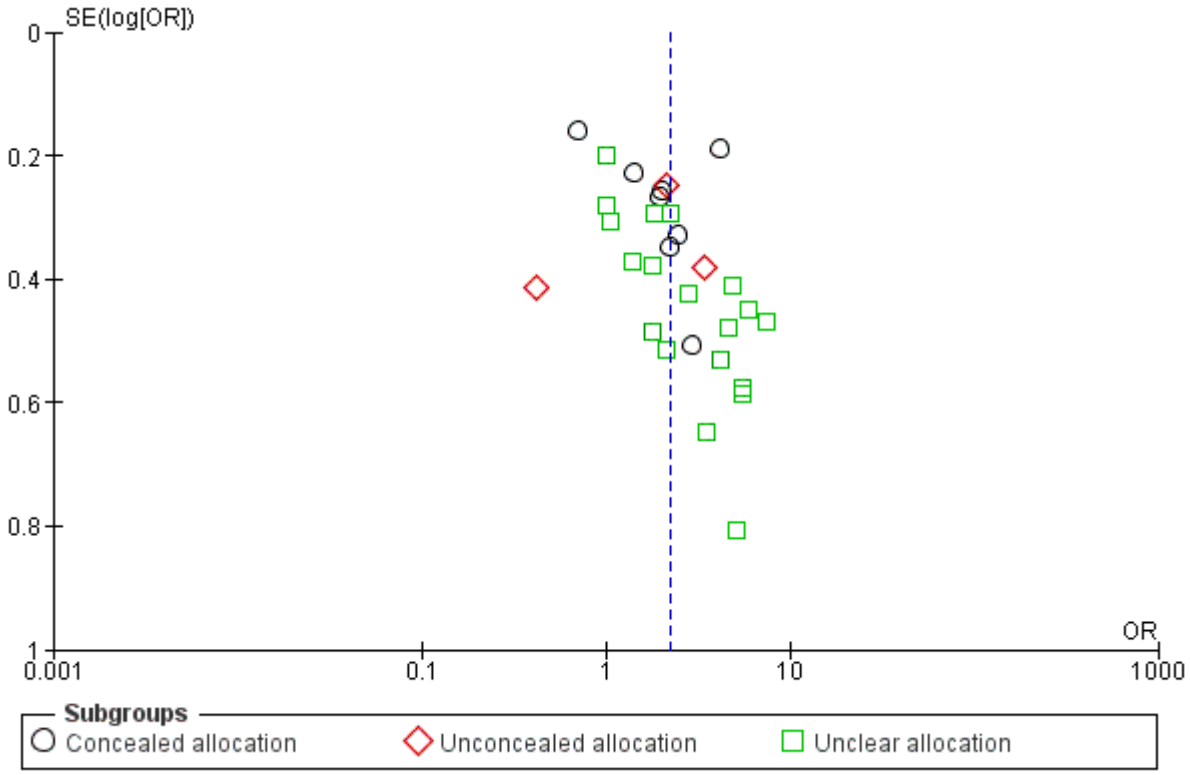
Eighteen (60%) studies did not report any information on the allocation of the sequence generation and in 5 (17%) studies the sequence was reported as 'not allocated' (Figure 16).

In order to generate unpredictable and unbiased sequence, 7 (23%) studies reported 'adequate' concealment of the allocation sequence mainly using opaque sealed envelopes and by involving personnel in the enrolment phase that were unaware of upcoming assignment of participants to treatments.

For instance, the study by Albrecht et al[72] published in 2011 reported that the intervention sets were prepared by the hospital's pharmacy and by independent personnel not involved in the study. Similarly, in the trial by Kim et al[62] published in 2011, only the independent staff could manage a matching list between study identification number and hospital number and the data were revealed to other investigators once recruitment and data collection were completed.

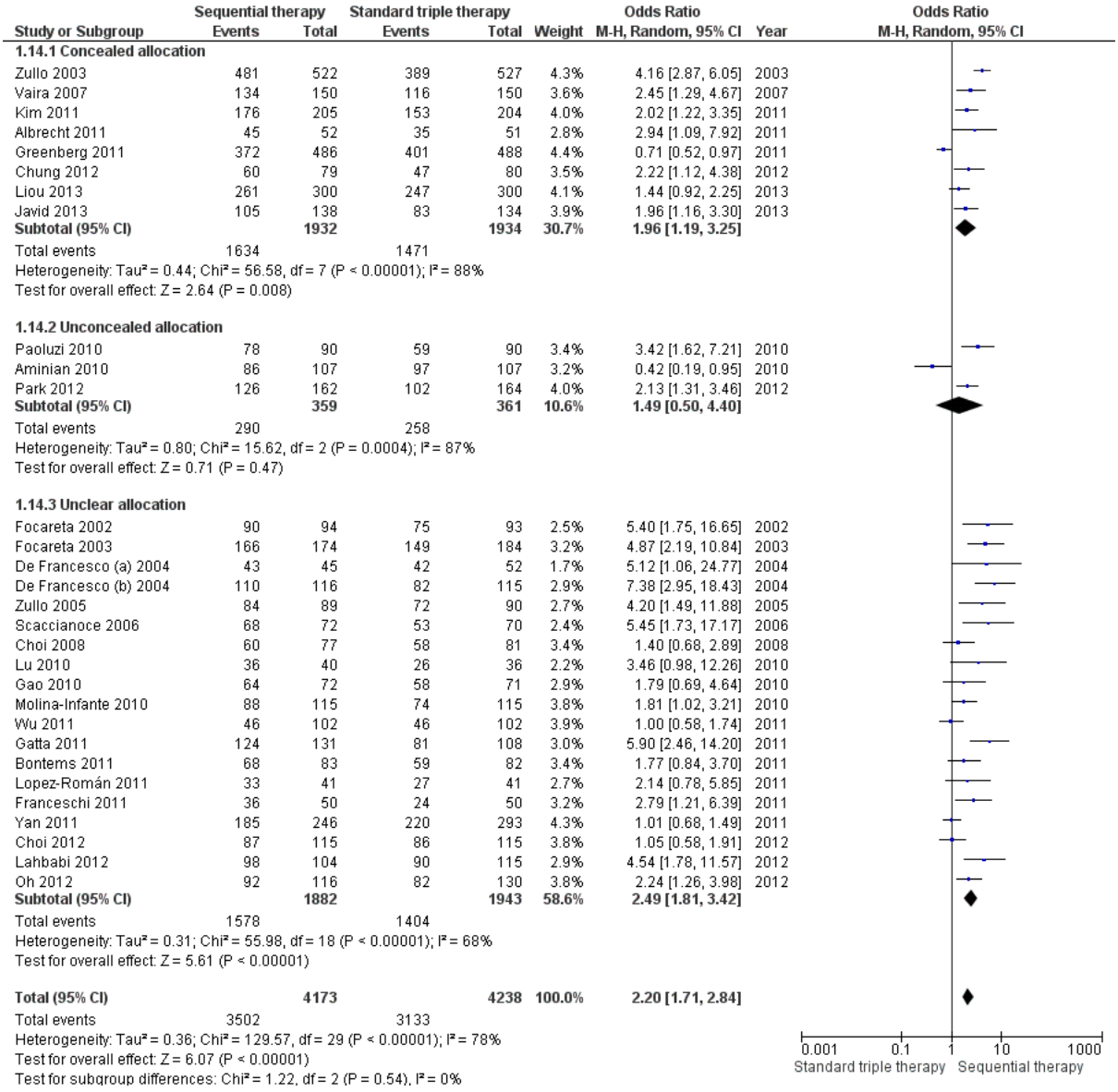
The Figure 18 below shows that outcomes were unlikely to be influenced by any potential selection bias.

Figure 18. Funnel plot of comparison: SEQ vs. STT, outcome: Allocation concealment



Also, the forest plot comparing the interventions assessed for the outcome 'allocation' (Figure 19) showed that patients could benefit more from the SEQ versus the STT in both the subgroups' analyses where the allocation was either concealed or unclear although a higher effect size was observed among studies with unclear allocation (OR 2.49 vs. 1.96). Additionally, no significant differences were found between treatments in studies were allocation was unconcealed (OR= 1.49; [95%CI= 0.50-4.40]; p<0.5).

Figure 19. Forest plot of comparison: SEQ vs. STT, outcome: Allocation concealment



4.3.2. Blinding

Regarding masking, 14 (47%) studies were adjudicated a 'high risk' category as authors reported either that the trial was not blinded or the design of the study was 'open-label'. Similarly, other 14 studies under the category 'unclear risk' either did not report any information regarding masking or authors stated that only the investigators (but not the patients), were blinded to the treatment allocation in that case studies were tracked single-blinded (Figure 16).

Two studies[57, 72] were categorised 'low risk' given authors stated a 'double-blind' design was used with placebo during 3 days after completion of the STT. Being only two studies, no subgroup meta-

analysis was performed with them as established in the protocol. The eradication rates were calculated as 89% and 86% in the SEQ arms and 77% and 69% in the STT arms in the studies by Vaira et al and Albrecht et al respectively.

It should nonetheless be noted that the number of studies that were not blinded was a priori due to the design of the sequential regimen itself, where most of the times two drugs were used in the initial phase and three drugs during the second phase of treatment (as per protocol). As per such way of administering drugs in both of the regimens, participants could not be blinded in practical terms.

However, the funnel plot below showed that outcomes were not influenced by bias associated with blinding, (Figure 20) and SEQ was reported to be more effective than STT regardless of the masking of the studies (OR= 2.43; [95%CI= 1.65-3.59]; p<0.001; Figure 21).

Figure 20. Funnel plot of comparison: SEQ vs. STT, outcome: Masking

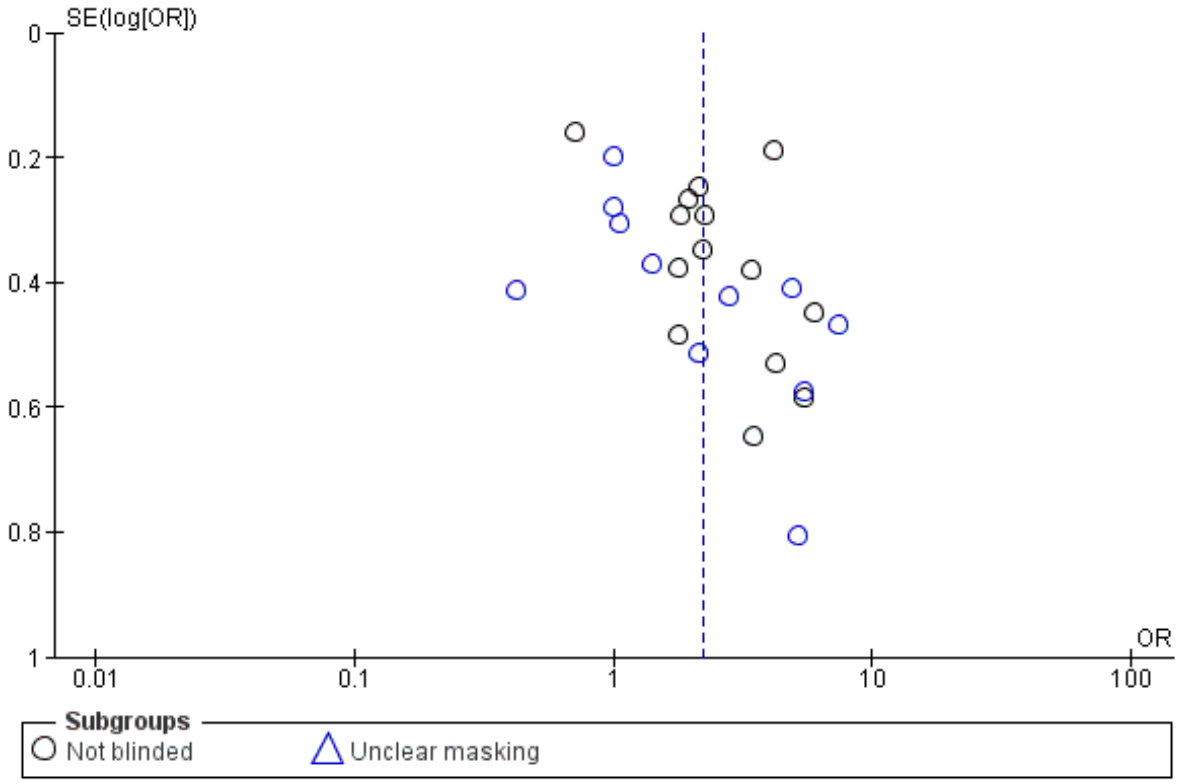
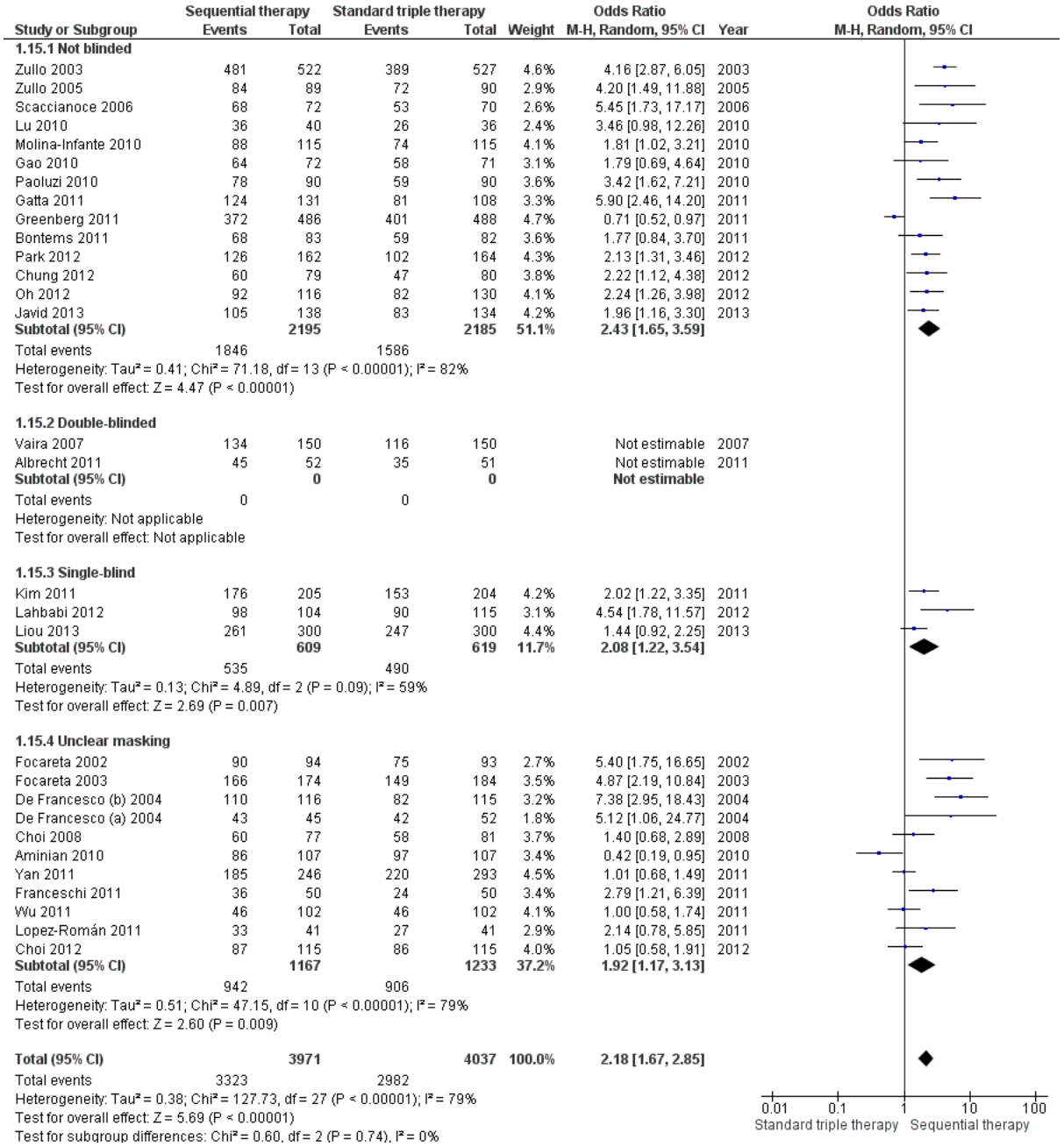


Figure 21. Forest plot of comparison: SEQ vs. STT, outcome: Masking



4.3.3. Incomplete outcome data

Primary outcomes were correctly and consistently reported in the majority (75%) of the studies (Figure 15). Attrition bias was reported in 2 of the 7 studies in abstract form[66, 73] accounting for around 1,709 patients, which represented 19% of the total of the randomized population in current meta-analysis.

Indeed, information related to the medical condition at baseline, sex ratio, average age of the population, PP sample size, incidence of AEs or antibiotic resistance were scarcely described in the reports of abstracts of Congresses.

No difference in the number of excluded participants or drop-out was noted between arms across included studies.

4.3.4. Selective reporting

Five (18.5%) studies out of 30 evaluating antibiotic resistance; 4 reported the different cut-off points for isolates assessed to nitroimidazole, clarithromycin, and amoxicillin.

Minimal inhibitory concentrations to consider resistance were reported as ≥8 μg/mL for metronidazole, ≥1 μg/mL for clarithromycin and between 0.5 and 1 μg/mL for amoxicillin across all 4 studies. The remaining study was an abstract and the information was not available and could neither be retrieved from the authors.

Therefore, bias associated with selective reporting of the aforementioned outcome measure seemed likely.

4.3.5. Other potential source of bias

Twenty-three (77%) studies were in complete article form. No bias due to the status of the publication was able to influence outcomes (Figure 22). SEQ was significantly more effective than STT in studies presented as full articles and abstracts but the effect size reported was slightly greater among trials presented as abstracts (OR 2.47 vs. 2.12; Figure 23).

Figure 22. Funnel plot of comparison: SEQ vs. STT, outcome: Publication Format

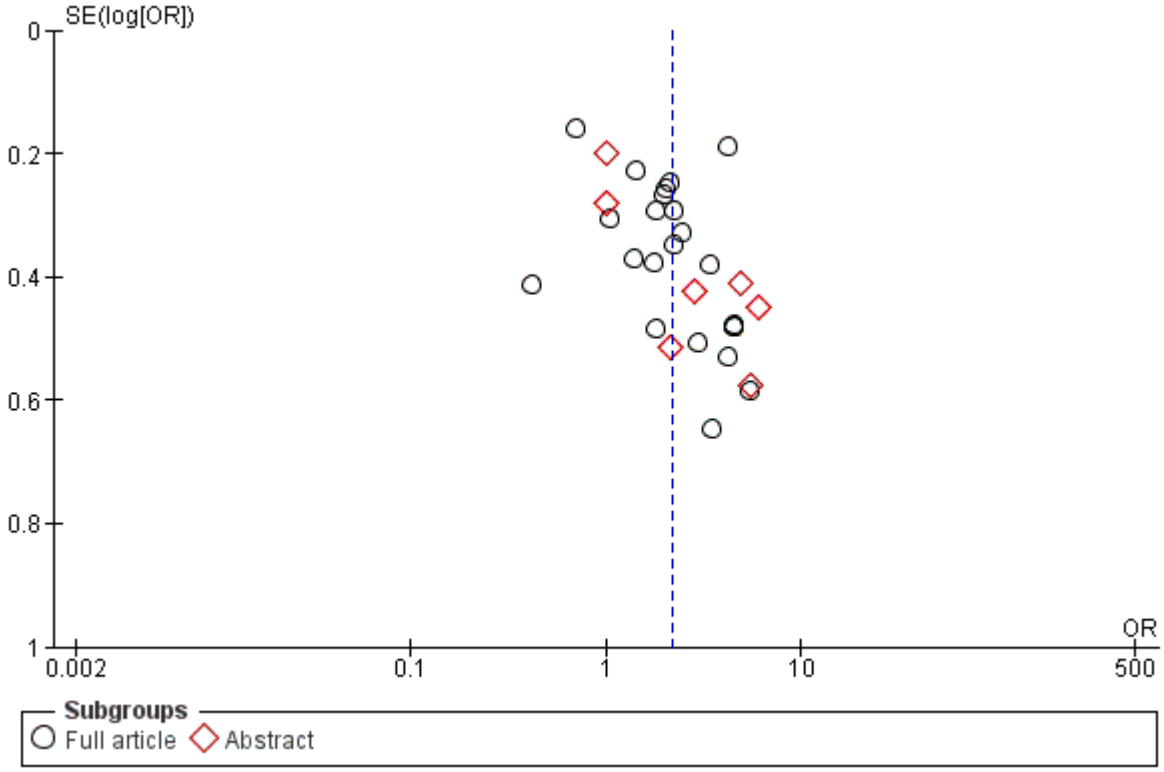
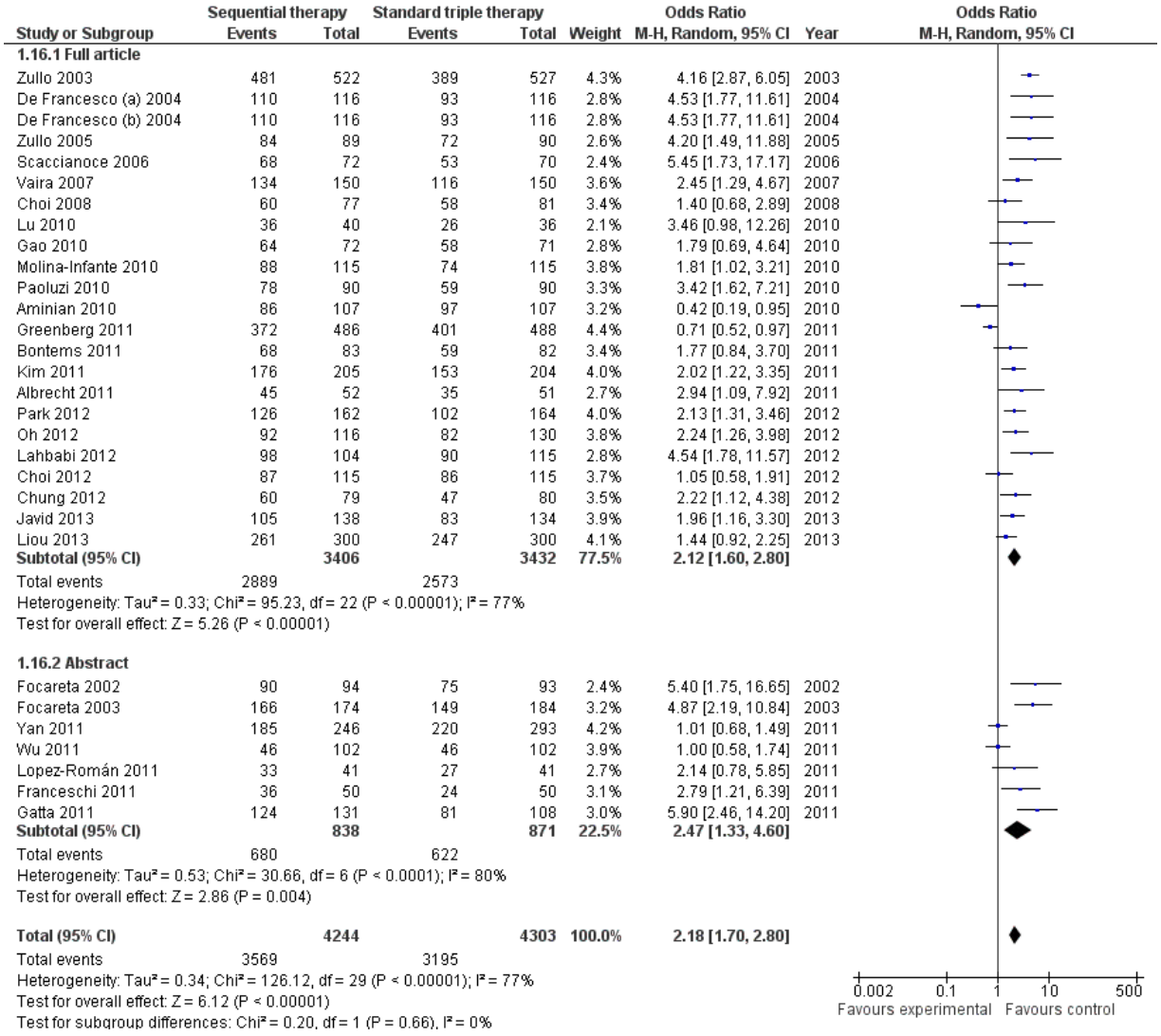


Figure 23. Forest plot of comparison: SEQ vs. STT, outcome: Publication Format



5. Discussion

Multiple treatments have been suggested for *H. pylori* infection and that has been frequently reported and discussed in the literature. Despite the large number of studies performed in the last two decades, no optimal first-line eradication regimen has been yet defined.

There could be many explanations but mainly efficacy, cost-effectiveness, toxicity and ease of administration of drugs as well as antibiotic resistance, have been reported among current challenges that need to be overcome.

Main findings of the present systematic review

The primary aim of this systematic review was to evaluate the efficacy of 10-day SEQ versus STT addressed in the available published RCTs. The secondary objective was to compare the incidence of AEs.

The screening and full-text assessment of citations resulting from both the electronic and manual searches yielded the inclusion of 30 RCTs. All studies addressed treatment and compared 10-day SEQ versus 7, 10 or 14-day STT.

From the included studies, 7 (23%) were published as abstracts from Congresses and/or Conferences and the subgroup analysis showed that although heterogeneity was high (I^2=77%) among these studies, no bias was associated with the format of the publication neither the quality items assessed in the total of studies. This gave certain robustness to the findings of this systematic review.

Among the other subgroup analyses, a high proportion of studies were published in Italy (n= 10), many others were published after 2008 (n= 22), very little evidence was published in children populations (n= 3), and pre-treatment antibiotic susceptibility and/or resistance were barely mentioned (n= 5).

Overall, the efficacy of 10-day SEQ was higher than treatment with 7 or 10-day STT but no differences were found when 10-day SEQ was compared with 14-day STT.

Overall efficacy of SEQ vs. STT

The efficacy endpoint of interest collected was the *H. pylori* ITT eradication rate. From the 30 included studies and a total of 8,878 patients, a mean *H. pylori* cure rate of 84% in the SEQ arm vs. 74% in the STT arm was calculated.

The overall analysis proved a significantly higher efficacy of the SEQ over the STT supporting findings from previously published pooled data-analyses.[12, 23, 29, 31, 77-80] However, eradication rates in both SEQ and STT arms still remained lower (80-85%) than the optimal eradication levels (> 90-95%) required for microbial infections.[22]

Findings of the current meta-analysis also showed a decreased efficacy of SEQ (and potentially STT) therapies through the last years. In fact, substantially decreased eradication rates (lower than 80%) by triple therapies have been reported in Europe,[21, 48, 81] Asia,[82] United Sates[18] and Canada,[83] and likewise some authors have proposed an eradication failure rate greater than 20%.[84]

Lack of optimal treatment effect has been mainly attributed to antibiotic resistance. Many studies addressing eradication therapies for *H. pylori* infection have been published and included in new systematic reviews and meta-analyses.[17, 85]

Additionally, the success or failure of antibiotic regimens has been also associated to a number of different factors such as: number of antibiotics used, poor compliance, type of underlying disease such as PUD or NUD, shorter vs. longer STT duration (7 vs. 10 vs. 14 days), drug-related AEs, previous stomach bacterial load, bacterial virulence (Cag A status), tobacco use, age of the population, geographical region, or any other variable that could predict or influence the treatment outcome.[86]

In the present meta-analysis, several subgroup analyses were performed in order to assess the impact of such factors in the efficacy of both SEQ and STT. Candidate hypothetical theories are presented in order to explain such differences.

Variables influencing efficacy of SEQ

Geographic region

A previous review published in 2010 by Gisbert et al[31] showed that almost all studies comparing SEQ and STT therapies were performed in Italy, contributing to a lack of validation of findings in other settings.

In the current systematic review, 11 studies were performed in Italy and all of them showed a significant and clear advantage of SEQ over STT. On the other hand, 9 (out of 17) studies published out of Italy could not find differences between therapies and those were mostly conducted in Asia where antibiotic resistances, especially nitroimidazole resistance, have been reported higher than in other settings. For instance, the World Gastroenterology Organization Global Guidelines (2011) reported that in China (2007) metronidazole and clarithromycin resistance rates reached 76% and 28% respectively. Similarly, in Iran (2007) metronidazole and clarithromycin resistance rates were

73% and 9% respectively.[87] Aforementioned figures could explain the results differences in studies conducted in such countries regarding data of Italian and in general European studies.

Moreover, the subgroup analysis showed that all studies performed in Italy but 3 were published before 2008. Now, it could be argued that opposite phenomenon would be taking place in Italy given to the lack of findings' validation in such setting through the most recent years.

In fact, as some authors have already postulated, the origin of the studies should not be considered that relevant. For the purpose of efficacy and antibiotic resistance analyses, it would be more reasonable to consider the site of the studies as a surrogate factor for a given pattern of efficacy (or resistance) rather than a direct predictor of efficacy outcome.[88, 89]

Publication date

In line with the geographic region factor, if one takes into account the year of publication of the included studies, a distinct trend toward a lower efficacy of SEQ was shown in studies published after year 2008 and mainly in those published out of Italy (OR 1.55 vs. 3.63). As much as 9 studies published after 2008 could not find differences between SEQ and STT whereas almost all Italian studies (but one) performed in 2007 and before showed that patients receiving SEQ could benefit more than those receiving STT.

Currently, a trend to lower *H. pylori* cure rates following STT has been suggested but more recently, an epidemiological analysis of all published Spanish trials revealed a rather constant eradication rate over the years.[17] Published literature on the topic argues antibiotic resistance might be one of the most relevant factors mediating therapies' efficacy trend through the years.

Also, another theory advocated higher STT cure rates within early studies due to investigators involving initially more patients with peptic ulcers, in which eradication rates were reported better than in patients with NUD.[90] This statement could be potentially extended in the same way to justify SEQ eradication trends. However, data from present meta-analysis indicated that SEQ was less influenced by the type of the disease (PUD vs. NUD) than STT did.

Confounding variables

It is worth noting that from the results of this meta-analysis, it was not possible to determine the reason why studies published before 2008 resulted in higher treatment efficacy following SEQ (92%) compared with those published after 2008 (80%). The cause could depend on the modulating effect of either the geographic region or the publication date of the included studies.

As mentioned, only Italian studies were published before year 2008 and treatment success or failure only relied on these studies. Additionally, no study was reported to be published in year 2009, which added a cut-off point to the time trend-line making it challenging to interpret.

In order to investigate how these studies' characteristics might be associated with the intervention effects, meta-regression was considered as an extension to subgroup analyses as it allows the effects of multiple factors (geographic region and publication date) to be investigated simultaneously.

However, it was finally decided not to perform the aforementioned analysis. As per the recommendation in *The Cochrane Handbook of Interventions*,[33] meta-regression should not be considered when there are fewer than 10 studies in a meta-analysis. In our case, 11 and 9 studies respectively were pooled in each of the subgroup analyses.

Medical condition

Dyspepsia (functional or non-investigated) is a common condition, and no therapy has been yet described in treating effectively this disorder. Yet before SEQ was postulated, several investigations attempted to identify the relationship between *H. pylori* and NUD and whether eradication of the infection produced relief of dyspeptic symptoms in patients with NUD. The majority of the studies yielded with poor or inconclusive results.[91, 92]

Findings of the current meta-analysis suggest that eradication rates following SEQ were equal in NUD and PUD patients (86%). However, when both of the 2 therapies were compared, SEQ was statistically more effective than STT among patients with PUD, whereas differences between the 2 regimens could not be demonstrated among patients with NUD, probably due to the fact that STT obtains higher eradication rates in PUD than in NUD, which would reduce the difference with SEQ in those patients.

As reported in previous studies,[29, 50] the fact that eradication rates in both PUD and NUD patients following SEQ were similar suggests that the sequential scheme might overcome differences of patients´ baseline medical conditions in a similar manner, or that the underlying disease itself is not a moderator neither a predictor of the treatment outcome.

STT length

In order to support and reinforce the curative effect of STT some studies did focus on investigating treatment durations. It has been postulated that longer regimens, for example extending STT to 14 days might result in higher cure rates.[93-96]

In the current meta-analysis no differences in terms of efficacy between SEQ and STT regimens were found when STT lasted 14 days. Given these results, the final decision regarding the administration of one or the other will depend on the safety and cost-effectiveness of both therapies in each context.

PPI type

Eradication rates with SEQ were higher than with STT in patients using both new generation and old generation PPIs. Findings of the current meta-analysis in SEQ treatment appeared to show an increase in efficacy with new generation PPIs when compared to old generation PPIs as described for STT in former meta-analyses.[97] [98]

Moreover, within the subgroup analysis, the difference in efficacy between therapies was even greater when using new generation PPIs.

Concerning dosage, almost all studies but 2 used the different PPIs at standard doses. One study[54] used esomeprazole 40 mg twice daily. *H. pylori* cure rate was reported greater with SEQ than with STT (95% vs. 75%, respectively) and was among the highest eradication rates (95%) achieved with SEQ across the included studies.

Unfortunately, no further assessment regarding dosage could be performed given no more of the included studies used high-dose PPIs. Nonetheless former meta-analyses did already advocate the clear benefit of high-dose PPIs compared to standard-dose for treatment of *H. pylori* infection with 7 days STT.[99]

Antibiotic resistance

Antibiotic resistance was scarcely reported: only 5 studies reported clarithromycin, and 4 reported nitroimidazole resistances. This represented a major limitation of current systematic review due to the lack of reporting of reliable, consistent and updated information regarding the prevalence of antibiotic susceptibility and resistance within the primary included RCTs. However, although none of the subgroups showed significant differences between the two treatment arms, the results indicated that particularly patients with single clarithromycin resistance could benefit more from SEQ than from STT.

Antimicrobial resistance has been considered the main responsible factor for the low efficacy of standard triple therapies and for the decrease in eradication rates with SEQ.[100-102] In the current meta-analysis, 3 studies published in Italy by Zullo et al, Vaira et al and Gatta et al in 2003, 2007 and 2011 respectively showed that SEQ was significantly more beneficial than STT mainly in those

patients with bacterial resistance to clarithromycin. However, our meta-analysis has not been able to demonstrate such good efficacy figures for these patients.

Moreover, the difference between SEQ and STT did not reach statistical significance in patients with clarithromycin resistant strains although the difference in eradication rates between SEQ and STT in the current meta-analysis (30%) was reported similar to the difference in eradication rates between SEQ and STT in two previous meta-analyses (57% and 37% respectively) published in 2008 by Jafri et al[77] and in 2009 by Gatta et al.[30] Such differences between treatments could be due to a small sample size.

Age of the population

Only 3 RCTs assessed SEQ vs. STT in children. Treatment with SEQ was found more beneficial than with STT (85% vs. 71%) and similar to the adult population (84% vs. 74%, respectively).

Previous data from meta-analysis showed similar results,[30, 79] although as it was the case for adult patients, eradication rates with SEQ did not achieved the desired level of success.

Safety

Safety was assessed through the incidence of AEs of included studies. The main category reported was gastrointestinal distress such as abdominal pain, diarrhoea, nausea, glossitis and vomiting.

From the studies addressing tolerance and compliance, the overall incidence of AEs with SEQ and STT was reported equal (18.5% in both arms). The interruption of treatment due to AEs was also similar between treatment arms (near 1% with SEQ and 1.5% with STT).

Findings from the present review support data from previous meta-analyses,[30, 31, 77] where AEs as well as compliance rates were found comparable between both regimens.

Quality of the methodology of included studies: limitations and advantages

Intention-to-treat reporting

For the meta-analysis purposes, ITT rates were reported as per primary author´s statements. That is, all patients after randomization were accounted for analysis and any clinical trial´s complications such as non-compliance, withdrawals, protocol deviations and anything happening after randomization where not considered.[103]

In the present meta-analysis, complete outcome data were available in all included studies but 2 demanding situations were noted when collecting 'figures'. First, in some studies the number of

patients randomized to each of the treatment arms was not provided,[66, 73] therefore the ratio specifying the number of patients cured over the total number of patients randomized to the specific treatment arm had to be estimated from the provided percentage of patients cured. Estimated figures were not always exactly matching provided percentages.

Secondarily, it was also noted that although ITT rates were used as per definition above, from the perspective of the systematic review this could not be confirmed in most of the cases. The reason is that RCTs did not report reliable, complete and uniform definitions regarding participation rates within the study flow diagram. And thus, proportions of patients allocated to one treatment arm or another might be responding to different participation definitions. On the other hand, authors of primary studies might be reporting proportions without explicitly specifying to which participation definition they were initially referring.

Reporting of baseline characteristics by treatment arm vs. not reporting findings by treatment arm

Three studies assessed the efficacy of SEQ versus STT in both NUD and PUD patients without reporting results independently whereas NUD and PUD patients were assessed independently in eight studies each. Data from these 3 studies could not be included in the subgroup meta-analysis. Authors were contacted but these results were not provided.

Masking of personnel and participants

In this systematic review, most of the studies were not blinded (neither single nor double-blinded) and this could be considered as reducing considerably the quality of the included studies. However, it is generally accepted that *H. pylori* eradication is not affected by blindness as it is unlikely that the placebo effect would have an effect on the tests performed to confirm eradication nor in the bacteria itself.

Furthermore, unmasked studies give a better estimation of the efficacy in clinical practice as it could be speculated that the more complex sequential regimen may affect compliance and therefore treatment success.[104]

Sample size

In the meta-analytic context, larger sample sizes are better to increase the confidence of an estimate. In this meta-analysis, as much as 13 studies (46%) had a sample size of less than 100 patients at randomization. Post-hoc sensitivity analyses did not show to improve the overall effect size of SEQ when sample sizes of these same studies were doubled in each of the arms. This conferred robustness to the results of the meta-analysis.

Recommendations, other treatments for *H. pylori* eradication and further research

At present STT is recommended as first line therapy for the eradication of *H. pylori* in several countries.[2] On the other hand, many studies have confirmed a better efficacy of SEQ especially when compared to 7 and 10 days STT. As previously mentioned, SEQ also reported encouraging results when used among clarithromycin-resistant populations.

STT can easily be converted into a non-bismuth quadruple therapy (either sequentially or concomitantly). Recently, a review evaluated findings of previous RCTs that had compared non-bismuth quadruple therapies with STT. Results showed that concomitant therapy is equally well tolerated than STT but still more effective.[105] Further to this research, an additional meta-analysis of RCTs comparing the concomitant and the standard triple therapy demonstrated non-bismuth quadruple (concomitant) therapy appeared to be an effective, safe, and well-tolerated alternative to STT and was reported less complex than SEQ.[106]

However, there is a renewed need to update findings of the aforementioned meta-analysis. More recent studies have evaluated the use of non-bismuth quadruple therapies (both sequential and concomitant regimens) in clinical settings with increased clarithromycin resistance rates and although differences did not reach statistical significance, there was a tendency towards better efficacy with concomitant therapy.[107-110]

As per findings of the current meta-analysis, it is clear that, overall, SEQ is a better strategy than STT in the majority of the settings assessed. However, further robust assessment should focus on investigating the definitive better efficacy of SEQ when compared with STT lasting 14 days.

In any case, the overall efficacy obtained with SEQ treatment in the meta-analysis was sub-optimal. Moreover, there was a trend towards a reduction of efficacy in SEQ over the years which does not generate good future perspectives for this strategy.

Further research would be also essential comparing non-bismuth quadruple therapies (concomitant) with SEQ, so that a clear recommendation could be made towards a generalized change in first-line *H. pylori* treatment.

6. Authors' conclusions

The present systematic review and meta-analysis provided further and robust assessment across a much broader range of patients comparing SEQ versus STT than in previously published reviews.

Findings showed a clear benefit of SEQ over STT (7 and 10 days) in treatment naïve *H. pylori* infected patients. It was also confirmed the higher efficacy of SEQ vs. STT among patients with clarithromycin bacterial resistance.

However, as it is clearly demonstrated that STT efficacy is sub-optimal, a generalized change in first line treatment needs to be considered especially in those settings where eradication rates with STT have been reported lower.

Given the results of the present meta-analysis, whether SEQ should be the substitute of choice will depend on specific characteristics of the regional context, and, more importantly, the efficacy obtained with other proposed treatments such as non-bismuth quadruple concomitant regimen.

7. Conclusiones

La presente revisión sistemática y metaanálisis proporciona una evaluación más exhaustiva de la eficacia de la terapia secuencial frente a la triple terapia clásica en comparación con las revisiones publicadas anteriormente. Se ha incluido un número mayor de pacientes, actualizando los resultados previos y confiriendo mayor evidencia científica a dichos resultados.

El metaanálisis ha demostrado que la terapia secuencial es más efectiva que la triple terapia clásica (durante 7 ó 10 días) en el tratamiento de primera línea en pacientes infectados por *H. pylori*. Esta mayor eficacia no se evidenció, sin embargo, cuando la triple terapia se administraba durante 14 días.

También se ha confirmado el mayor beneficio de la terapia secuencial frente a la triple clásica entre aquellos pacientes con resistencia bacteriana a la claritromicina.

Sin embargo, también se ha puesto de manifiesto que la tasa de erradicación con la terapia secuencial es claramente subóptima y decreciente con el tiempo.

Según los resultados del presente meta-análisis, la cuestión sobre si la terapia secuencial debe sustituir a la triple terapia clásica como tratamiento de elección de primera línea para la infección de *H. pylori* dependerá de las características específicas en cada ámbito regional y, sobre todo, de la eficacia obtenida con otros tratamientos erradicadores como la terapia cuádruple concomitante.

8. Appendix: search strategy (electronic databases)

Ovid MEDLINE(R): searched from 1948 to May 2013

1. randomized controlled trial.pt.
2. controlled clinical trial.pt.
3. randomized.ab.
4. placebo.ab.
5. clinical trials as topic.sh.
6. randomly.ab.
7. trial.ti.
8. or/1-7
9. exp animals/ not humans.sh.
10. 8 not 9
11. Helicobacter pylori/
12. (H adj3 pylori).mp. [mp=protocol supplementary concept, rare disease supplementary concept, title, original title, abstract, name of substance word, subject heading word, unique identifier]
13. (helicobacter adj3 pylori).mp. [mp=protocol supplementary concept, rare disease supplementary concept, title, original title, abstract, name of substance word, subject heading word, unique identifier]
14. (C adj3 pylori).mp. [mp=protocol supplementary concept, rare disease supplementary concept, title, original title, abstract, name of substance word, subject heading word, unique identifier]
15. Campylobacter.mp. [mp=protocol supplementary concept, rare disease supplementary concept, title, original title, abstract, name of substance word, subject heading word, unique identifier]
16. Helicobacter Infections/
17. or/11-16
18. ((triple or standard) adj2 (regimen or therapy or treatment)).tw.
19. (sequential adj2 (regimen or therapy or treatment)).tw.
20. PPI.mp.
21. Proton Pump Inhibitors/
22. (Clarithromycin or biaxin or Claripen or Claridar or clarith or Crixan or Clacid or Fromilid or infex or klaricid or Klabax or Klacid or Vikrol).mp.
23. (amoxicillin or amoxycillin or actimoxi or almodan or amix or amox or amopen or amoram or amoxicot or amoxil or amrit or biomox or clamoxyl or dispermox or galenamox or larotid or moxatag or moxilin or p-hydroxyampicillin or penamox or polymox or respillin or rimoxallin or senox or sumox or Tormoxin or trimox or utimox or wymox or zoxycil).mp.
24. (Alphamox or Amocla or Amoksibos or Amoxiclav Sandoz or Amoxidal or Amoxin or Amoksiklav or Amoxibiotic or Amoxicilina or Apo-Amoxi or Augmentin or Bactox or Betalaktam or Cilamox or Curam or Dedoxil or Duomox or E-Mox or Enhancin or Gimalxina or Geramox or Hiconcil or Isimoxin or Klavox or Lamoxy or Moxilen or Moxypen or Moxyvit or Nobactam or Novamoxin or Ospamox or Panklav or Pamoxicillin or Panamox or Samthongcillin or Sinacilin or Tolodina or Yucla or Zerrsox or Zimox).mp.
25. nitroimidazoles/ or metronidazole/ or tinidazole/
26. nitroimidazole*.tw.
27. (Metronidazole or nabact or clont or danizol or edg dentalgel or elyzol or flagyl or gineflavir or metrocream or metrodzhil or metrogel or metrolotion or metrolyl or metronizole or metrotop or metrovex or metrozol or metryl or noritate or norzol or nydamax or obagi or protostat or rozex or satric or trichopol or tricom or trivazol or vandazole or vitazol or zadstat or zidoval).mp.

28. (Tinidazole or bioshik or fasigin or fasigyn* or tindamax or tricolam).mp.
29. or/18-28
30. 17 and 29
31. 10 and 30

EBM Reviews - Cochrane Central Register of Controlled Trials searched on 4th Quarter 2013

1. Helicobacter pylori/
2. pylori.mp. [mp=title, original title, abstract, mesh headings, heading words, keyword]
3. Helicobacter Infections/
4. or/1-3
5. ((triple or standard) adj2 (regimen or therapy or treatment)).tw.
6. (sequential adj2 (regimen or therapy or treatment)).tw.
7. PPI.mp.
8. Proton Pump Inhibitors/
9. (Clarithromycin or biaxin or Claripen or Claridar or clarith or Crixan or Clacid or Fromilid or infex or klaricid or Klabax or Klacid or Vikrol).mp.
10. (amoxicillin or amoxycillin or actimoxi or almodan or amix or amox or amopen or amoram or amoxicot or amoxil or amrit or biomox or clamoxyl or dispermox or galenamox or larotid or moxatag or moxilin or p-hydroxyampicillin or penamox or polymox or respillin or rimoxallin or senox or sumox or Tormoxin or trimox or utimox or wymox or zoxycil).mp.
11. (Alphamox or Amocla or Amoksibos or Amoxiclav Sandoz or Amoxidal or Amoxin or Amoksiklav or Amoxibiotic or Amoxicilina or Apo-Amoxi or Augmentin or Bactox or Betalaktam or Cilamox or Curam or Dedoxil or Duomox or E-Mox or Enhancin or Gimalxina or Geramox or Hiconcil or Isimoxin or Klavox or Lamoxy or Moxilen or Moxypen or Moxyvit or Nobactam or Novamoxin or Ospamox or Panklav or Pamoxicillin or Panamox or Samthongcillin or Sinacilin or Tolodina or Yucla or Zerrsox or Zimox).mp.
12. nitroimidazoles/ or metronidazole/ or tinidazole/
13. nitroimidazole*.tw.
14. (Metronidazole or nabact or clont or danizol or edg dentalgel or elyzol or flagyl or gineflavir or metrocream or metrodzhil or metrogel or metrolotion or metrolyl or metronizole or metrotop or metrovex or metrozol or metryl or noritate or norzol or nydamax or obagi or protostat or rozex or satric or trichopol or tricom or trivazol or vandazole or vitazol or zadstat or zidoval).mp.
15. (Tinidazole or bioshik or fasigin or fasigyn* or tindamax or tricolam).tw.
16. or/5-15
17. 4 and 16

CINAHL: searched up to May, 2013

S12	(S1 and S11)
S11	S2 or S3 or S4 or S5 or S6 or S7 or S8 or S9 or S10
S10	Tinidazole
S9	Metronidazole
S8	nitroimidazole*
S7	amoxicillin
S6	Clarithromycin

S5	Proton Pump Inhibitors
S4	PPI
S3	sequential and ((regimen or therapy or treatment))
S2	((triple or standard)) and ((regimen or therapy or treatment))
S1	Helicobacter pylori

EMBASE: searched from 1980 to May 2013

1. Clinical trial/
2. Randomized controlled trial/
3. Randomization/
4. Single-Blind Method/
5. Double-Blind Method/
6. Cross-Over Studies/
7. Random Allocation/
8. Placebo/
9. Randomi?ed controlled trial$.tw.
10. Rct.tw.
11. Random allocation.tw.
12. Randomly allocated.tw.
13. Allocated randomly.tw.
14. (allocated adj2 random).tw.
15. Single blind$.tw.
16. Double blind$.tw.
17. ((treble or triple) adj blind$).tw.
18. Placebo$.tw.
19. Prospective study/
20. or/1-19
21. Case study/
22. Case report.tw.
23. Abstract report/ or letter/
24. or/21-23
25. 20 not 24
26. Helicobacter pylori/
27. pylori.mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer]
28. Helicobacter Infections/
29. or/26-28
30. ((triple or standard) adj2 (regimen or therapy or treatment)).tw.
31. (sequential adj2 (regimen or therapy or treatment)).tw.
32. PPI.mp.
33. Proton Pump Inhibitors/
34. (Clarithromycin or biaxin or Claripen or Claridar or clarith or Crixan or Clacid or Fromilid or infex or klaricid or Klabax or Klacid or Vikrol).mp.
35. (amoxicillin or amoxycillin or actimoxi or almodan or amix or amox or amopen or amoram or amoxicot or amoxil or amrit or biomox or clamoxyl or dispermox or galenamox or larotid or moxatag

or moxilin or p-hydroxyampicillin or penamox or polymox or respillin or rimoxallin or senox or sumox or Tormoxin or trimox or utimox or wymox or zoxycil).mp.
36. (Alphamox or Amocla or Amoksibos or Amoxiclav Sandoz or Amoxidal or Amoxin or Amoksiklav or Amoxibiotic or Amoxicilina or Apo-Amoxi or Augmentin or Bactox or Betalaktam or Cilamox or Curam or Dedoxil or Duomox or E-Mox or Enhancin or Gimalxina or Geramox or Hiconcil or Isimoxin or Klavox or Lamoxy or Moxilen or Moxypen or Moxyvit or Nobactam or Novamoxin or Ospamox or Panklav or Pamoxicillin or Panamox or Samthongcillin or Sinacilin or Tolodina or Yucla or Zerrsox or Zimox).mp.
37. nitroimidazoles/ or metronidazole/ or tinidazole/
38. nitroimidazole*.tw.
39. (Metronidazole or nabact or clont or danizol or edg dentalgel or elyzol or flagyl or gineflavir or metrocream or metrodzhil or metrogel or metrolotion or metrolyl or metronizole or metrotop or metrovex or metrozol or metryl or noritate or norzol or nydamax or obagi or protostat or rozex or satric or trichopol or tricom or trivazol or vandazole or vitazol or zadstat or zidoval).mp.
40. (Tinidazole or bioshik or fasigin or fasigyn* or tindamax or tricolam).tw.
41. or/30-40
42. 29 and 41
43. 25 and 42

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