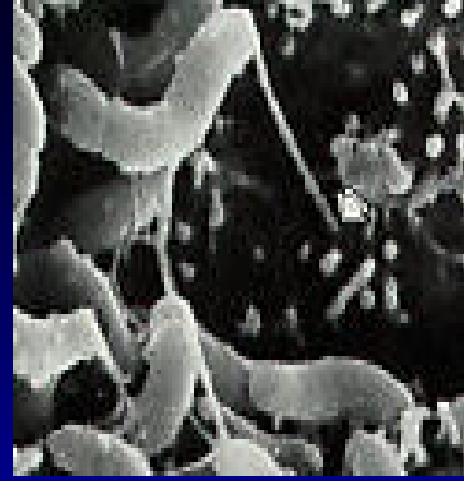


Helicobacter pylori: From its discovery to a revolution in gastroenterology

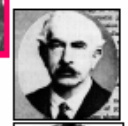
Y. Glupczynski

Laboratoire de Microbiologie
Cliniques Universitaires UCL de Mont-Godinne

Peptic ulcer and Nobel prize…over more than a century

- ✓ The presence of HCl in the stomach by W. Prout in 1823
- ✓ Vagus plays crucial role in gastric HCl secretion by I.P. Pavlov in 1895
- ✓ Gastrin is gastric HCl stimulant by J.S. Edkins in 1905
- ✓ *Dictum*; NO ACID NO ULCER by K. Schwartz in 1910
- ✓ Histamine as gastric HCl secretagogue by L. Popielski in 1916
- ✓ H_2-receptor antagonists by J.W. Black in 1972 and proton pump inhibitors by G. Sachs in 1980 showing strong inhibition of HCl secretion stimulated by various secretagogues and anti-ulcer efficacy
- ✓ *Helicobacter pylori* and its role in gastritis and peptic peptic ulcer disease by B.J. Marshall & R.J. Warren in 1983







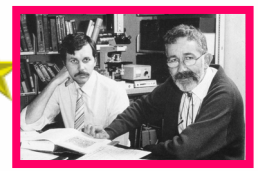



Fig. 1. Historical background; major discoveries in gastrology (star indicates Nobel prize)

H.pylori : Some key dates

Microscopic observation of spiral-shaped bacteria in stomach

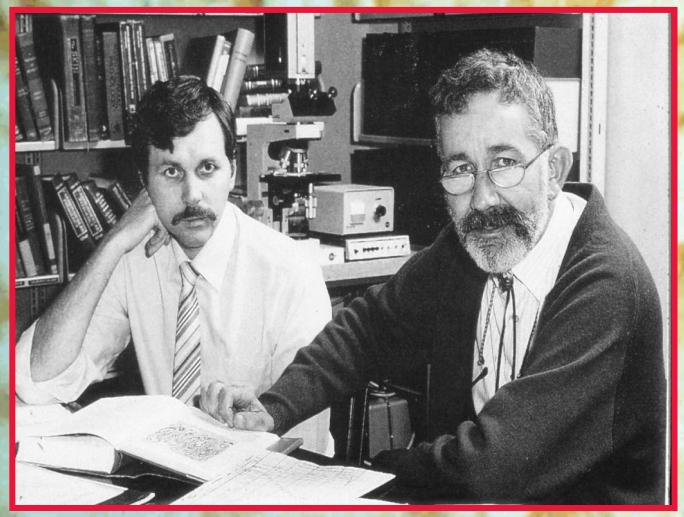
1893	Bizzozero	animal
1906	Krienitz	human
1975	Steer	human

Culture

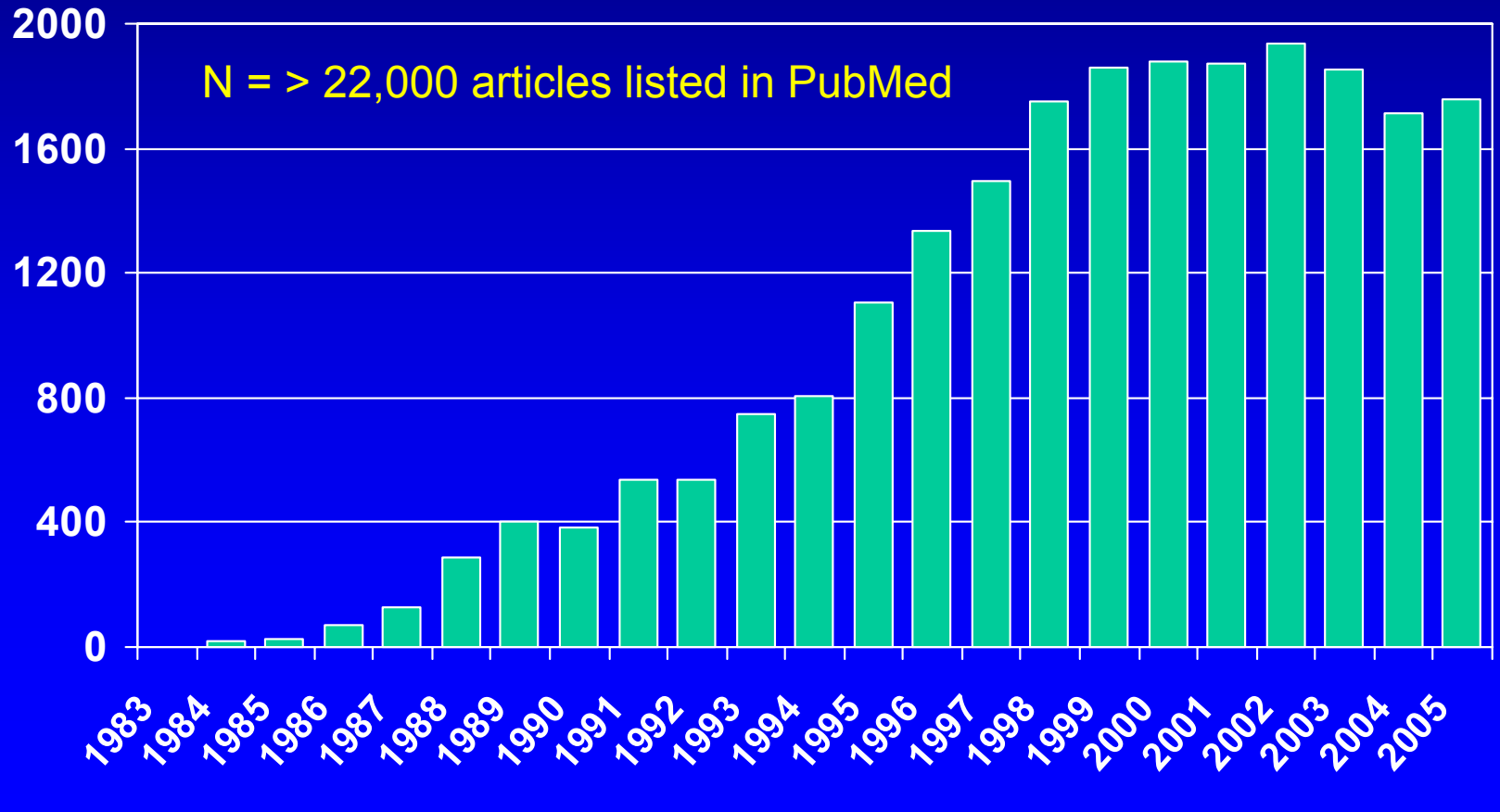
1982	Marshall	human

Nomenclature

1982	Marshall	*Campylobacter pyloridis*
1989	Goodwin	*Helicobacter pylori*



Evolution of Number of publications on *H.pylori* and gastroduodenal diseases



The milestone publications…

The Lancet · Saturday 16 June 1984

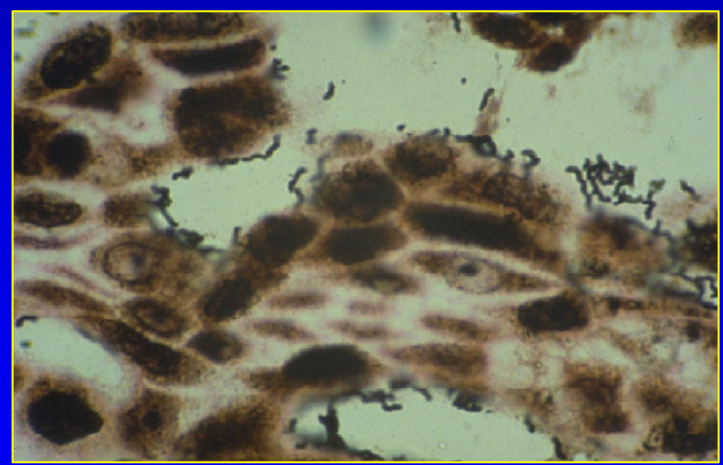
UNIDENTIFIED CURVED BACILLI IN THE STOMACH OF PATIENTS WITH GASTRITIS AND PEPTIC ULCERATION*

BARRY J. MARSHALL J. ROBIN WARREN

Departments of Gastroenterology and Pathology, Royal Perth Hospital, Perth, Western Australia

Summary Biopsy specimens were taken from intact areas of antral mucosa in 100 consecutive consenting patients presenting for gastroscopy. Spiral or curved bacilli were demonstrated in specimens from 58 patients. Bacilli cultured from 11 of these biopsies were gram-negative, flagellate, and microaerophilic and appeared to be a new species related to the genus *Campylobacter*. The bacteria were present in almost all patients with active chronic gastritis, duodenal ulcer, or gastric ulcer and thus may be an important factor in the aetiology of these diseases.

Organisms present in gastric biospies from:
- 100% of duodenal ulcers
- 80% of gastric ulcers
- 90% of active chronic gastritis
- 3% with no histological gastritis



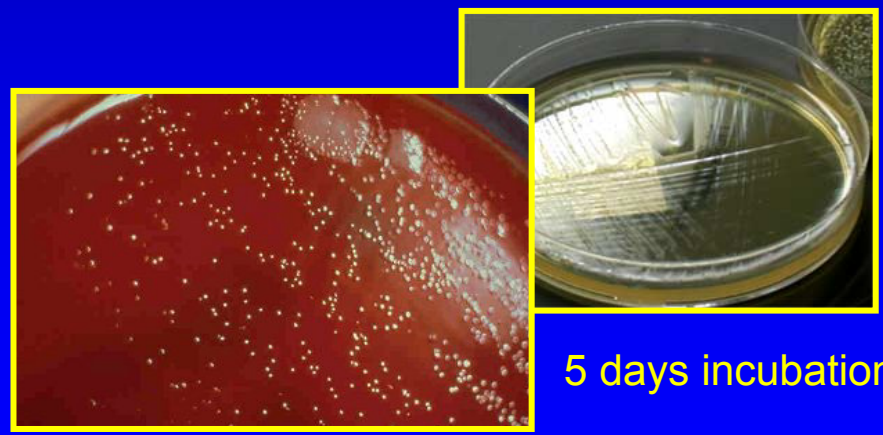
H. Pylori on gastric epithelial cells

Warren JR, Marshall BJ; Lancet i: 1273-5; 1983
Marshall B, Warren JR; Lancet i: 1313-5; 1984

Fruitless attempts to culture the elusive organism until …

Culture of gastric mucosa biopsies from 34 patients negative (after 48 h)

Royal Perth Hospital



5 days incubation

First cultures became positive only after prolonged Easter week-end

Resistance from the medical establishment…

2 Letter from the Gastroenterological Society of Australia*

GASTROENTEROLOGICAL SOCIETY OF AUSTRALIA

145 Macquarie Street,
SYDNEY. 2000

Telephone 27 3288

17th March, 1983

Dear Dr. Marshall,

I regret that your research paper was not accepted for presentation on the programme of the Annual Scientific Meeting of the Gastroenterological Society of Australia to be held in Perth in May, 1983.

The number of abstracts we receive continues to increase and for this Meeting 67 were submitted and we were able to accept 56.

There were a large number of high quality abstracts which made it extremely difficult to choose those which should be accepted for presentation, and as you know, this is now done by a National Abstract Selection Committee which reviews the abstracts without knowledge of the Authors concerned.

The National Programme Committee would like to thank you for submitting your work, and would hope that this might be re-submitted in the future, perhaps following critical review from your colleagues.

My kindest regards,

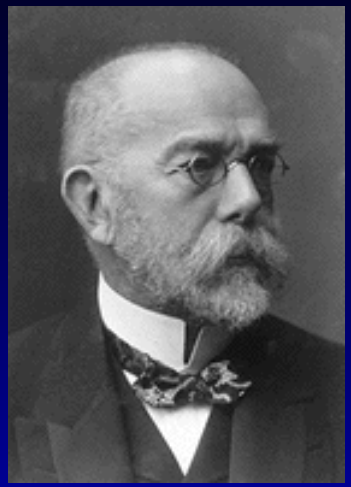
Yours sincerely,

for Terry D. Bolin,
Honorary Secretary.

Submission to the 1983 meeting of the Australian Gastroenterological Society was rejected….

…but was accepted to the 2nd International Microbiology Workshop on *Campylobacter* infections in Brussels in 1983

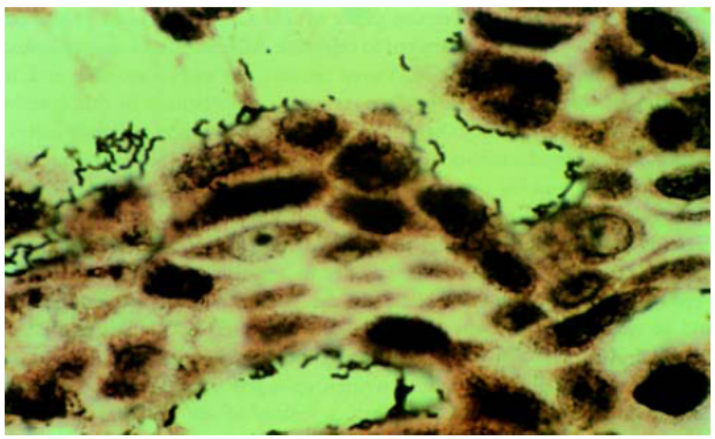
VRIJE UNIVERSITEIT BRUSSEL · SCIENTIA VINCERE TENEBRAS ·



Attempts to fulfil Koch's postulates (I)…

Self ingestion of a pure culture of *H. pylori* (10^9 organisms)

3 Silver stain of Marshall's gastric biopsy on Day 10 after ingesting *Helicobacter pylori*



Epithelial cells have rounded up in shape without intracellular mucin, and have many closely adherent black H. pylori *organisms. Reproduced from* Helicobacter *pioneers.*[3] ◆

- Day 5: halitosis, nausea, vomiting of acid free gastric juice
- Day 10: Endoscopy with biopsy Acute gastritis with many *H. pylori*
- Day 14: Rx with Bismuth/tinidazole resolution of symptoms

Marshall, Med J Aust 1985; 142: 436-9

Attempts to fulfil Koch's postulates (II)…

PROSPECTIVE DOUBLE-BLIND TRIAL OF DUODENAL ULCER RELAPSE AFTER ERADICATION OF CAMPYLOBACTER PYLORI

BARRY J. MARSHALL[1] C. STEWART GOODWIN[2]
J. ROBIN WARREN[3] RAYMOND MURRAY[1]
ELIZABETH D. BLINCOW[2] STEPHEN J. BLACKBOURN[4]
MICHAEL PHILLIPS[5] THOMAS E. WATERS[1]
CHRISTOPHER R. SANDERSON[1]

Departments of Gastroenterology,[1] Microbiology,[2] Histopathology,[3] and Pharmacy,[4] Royal Perth Hospital, Perth, Western Australia; and Center for Advanced Studies in Health Sciences, Curtin University, Perth[5]

Summary 100 consecutive patients with both duodenal ulcer and *Campylobacter pylori* infection were followed up to see whether eradication of *C pylori* affected ulcer healing or relapse. Patients were randomly assigned to 8 weeks of treatment with cimetidine or colloidal bismuth subcitrate (CBS), with tinidazole or placebo being given concurrently from days 1 to 10, inclusive. Endoscopy, biopsy, and culture were done at entry, in weeks 10, 22, 34, and 62, and whenever symptoms recurred. There was no maintenance therapy. *C pylori* persisted in all of the cimetidine-treated patients and in 95% of those treated with cimetidine/tinidazole, but was eradicated in 27% of the CBS/placebo group and 70% of the CBS/tinidazole group. When *C pylori* persisted, 61% of duodenal ulcers healed and 84% relapsed. When *C pylori* was cleared 92% of ulcers healed ($p < 0{\cdot}001$) and only 21% relapsed during the 12 month follow-up period ($p < 0{\cdot}0001$).

Cimetidine vs Bismuth (CBS) (8 Wks)
+ Placebo or tinidazole (10d)

Eradication of *H. pylori* :

increases healing of duodenal ulcer

92% (Hp -) vs 61% (Hp+)

decreases relapse rates at 12 months

21% (Hp-) vs 84% (Hp+)

Marshall BJ; Lancet 1988; 332;: 1437-42

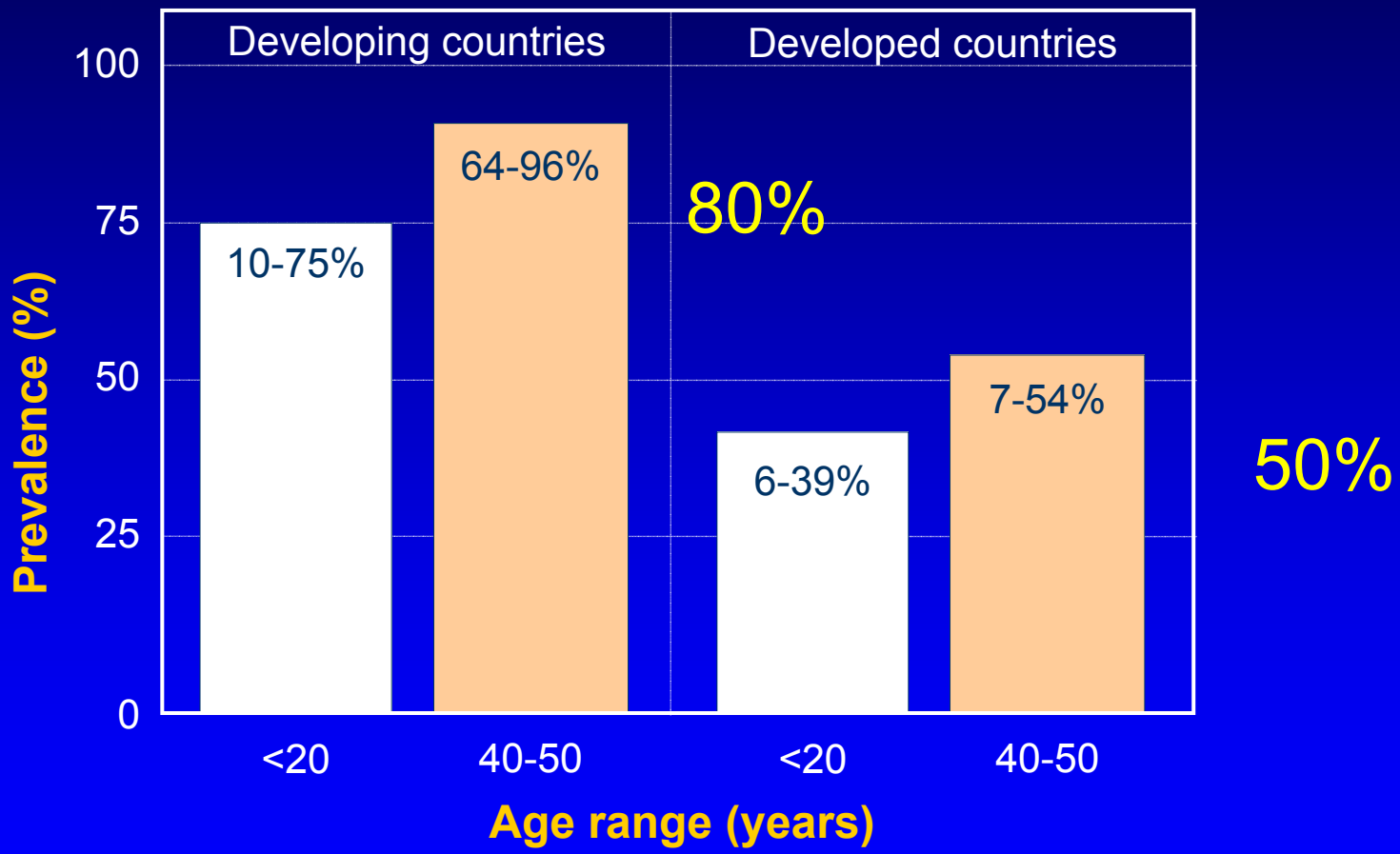
Hill causality criteria for association between *H. pylori* infection and gastroduodenal ulcer

- Strong association
- Temporal relationship
- Major effects of therapeutic intervention on outcome
- Biologic plausibility
- Biologic gradient ?
- But, no specificity of association

H.pylori : Epidemiology

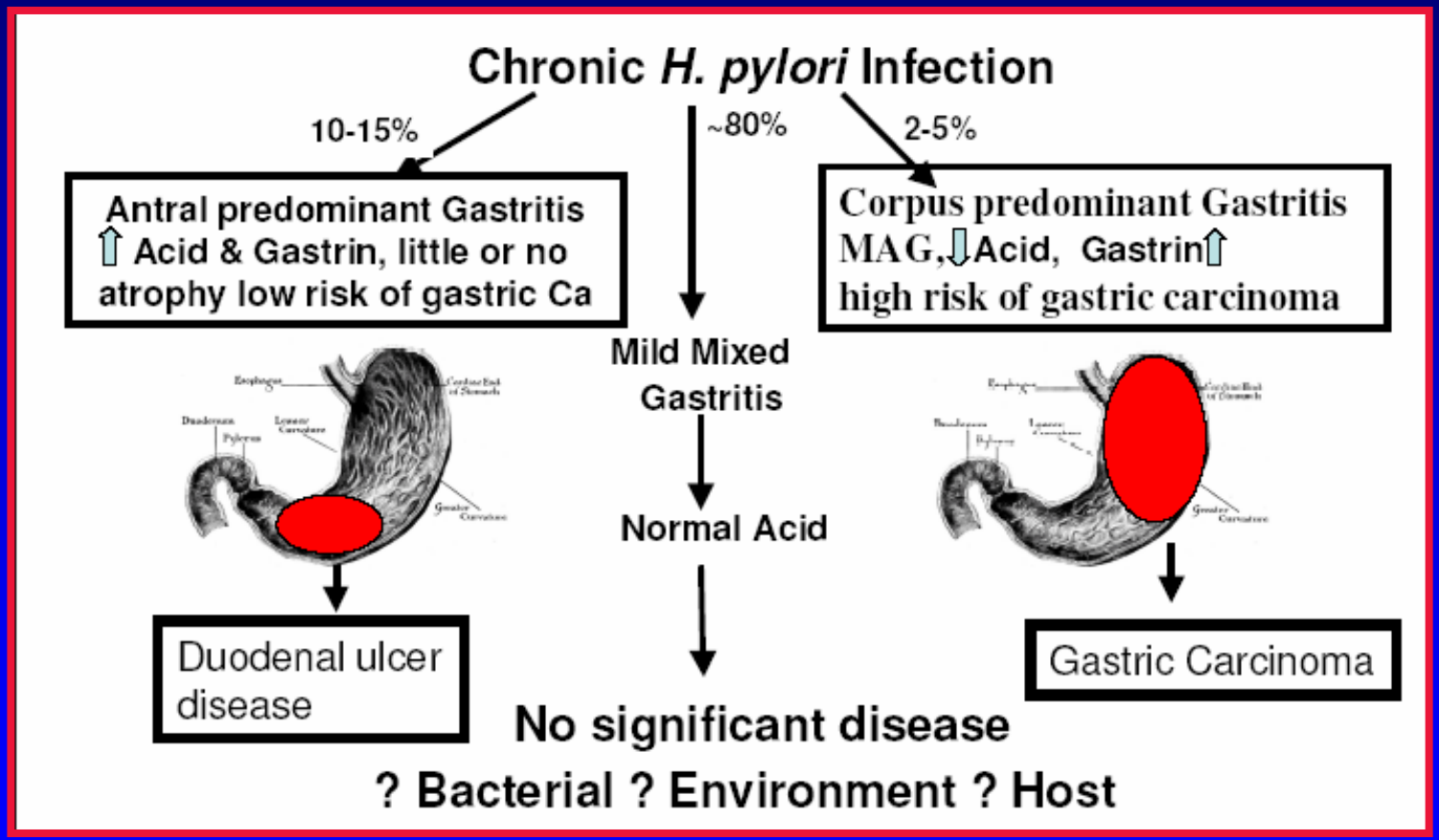
- Chronic infection
- Prevalence increases with age
  - incidence = 0.5-1% by person / year
- Infection acquired earlier in life and increased prevalence :
  - developing countries
  - populations with low socio-economic status
- Inter-human transmission
  - oral-oral and/or faecal-oral

Prevalence of *H. pylori* infection with age



Adapted with permission from Heatley-*Helicobacter pylori and Gastrointestinal Disease;* Oxford, UK: Blackwell Scientific Publications

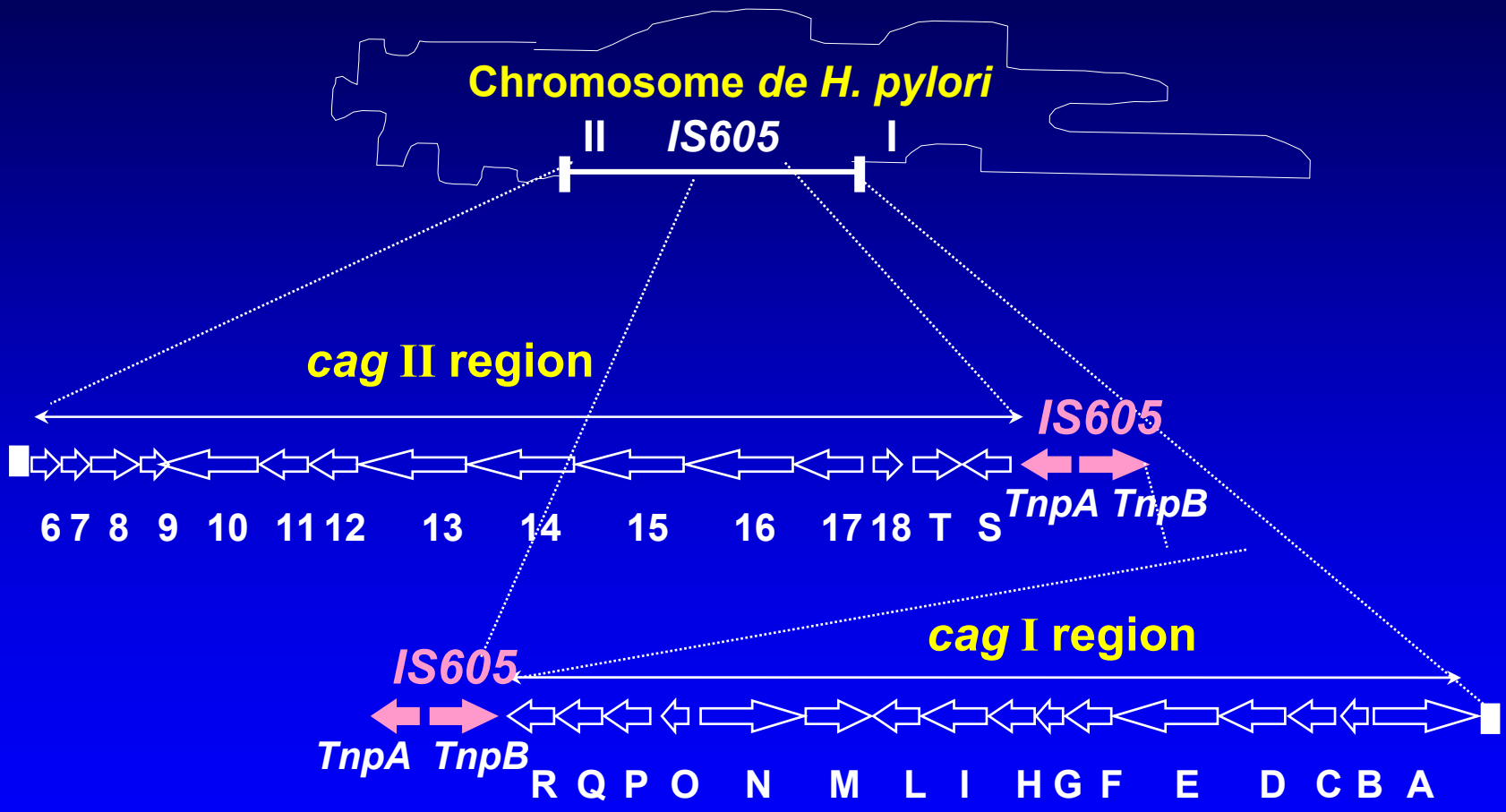
Divergent response to *H. pylori* infection



Major pathogenic factors of *H. pylori*

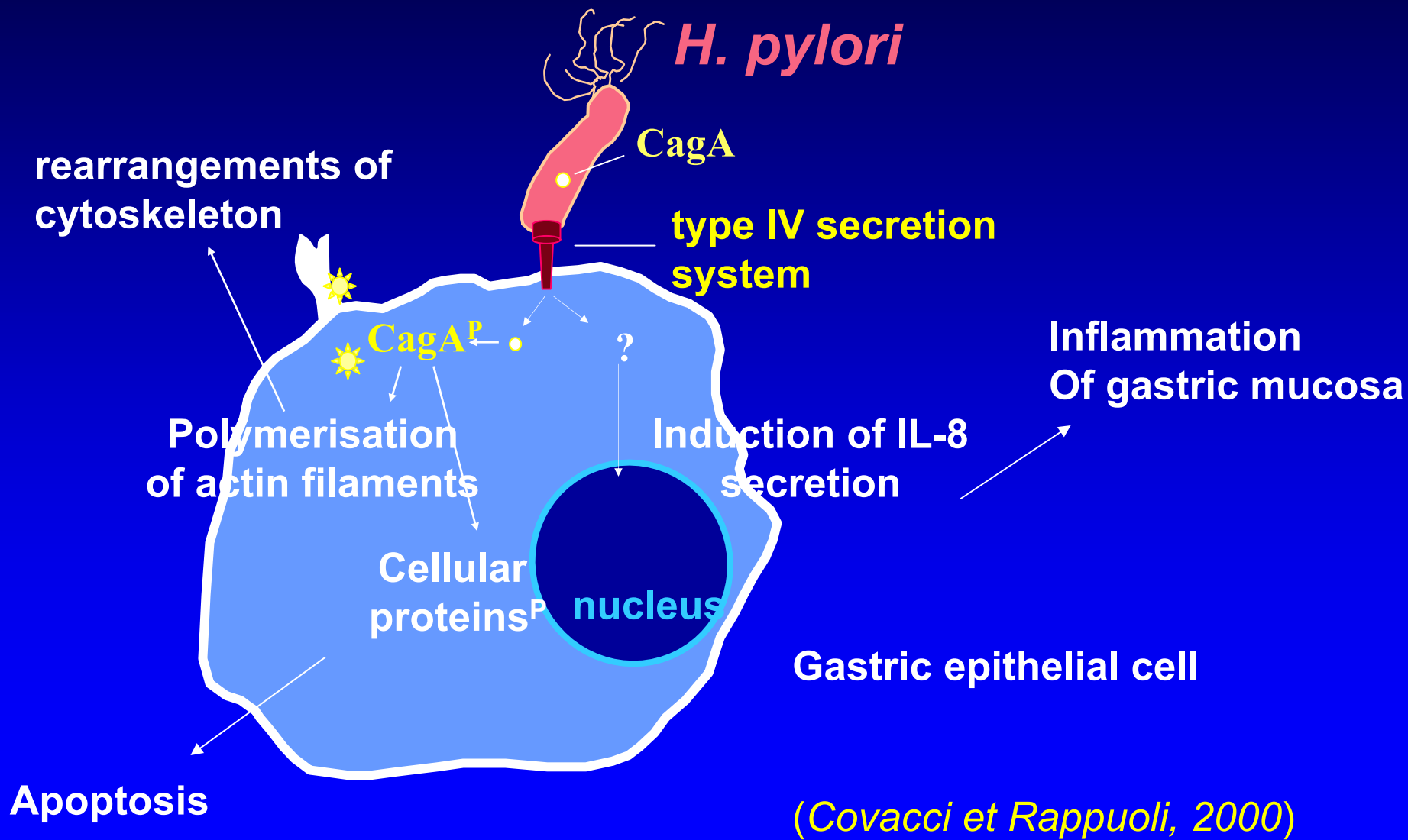
Virulence factors	Function	Present in all strains
Urease	Acid resistance; Nitrogen metabolism Escape to immunologic response Cytotoxic effect	Y
Flagella	Motility	Y
BabA	Adhesion to Lewis^{b} antigens	N
AlpA, AlpB	Adhesins (receptors not identified)	Y
Catalase Superoxide dismutase	detoxification (escape to immunologic response)	Y
Lewis^{X,Y} antigens (lipopolysaccharide)	Molecular mimicry (escape to immunologic response), adherence	N
VacA	Cytotoxicity	N
CagA	Immunodominant antigen unknown function	N
Cag pathogenicity island	Secretion system (type IV) ? Induction of inflammation	N

Cag pathogenicity island

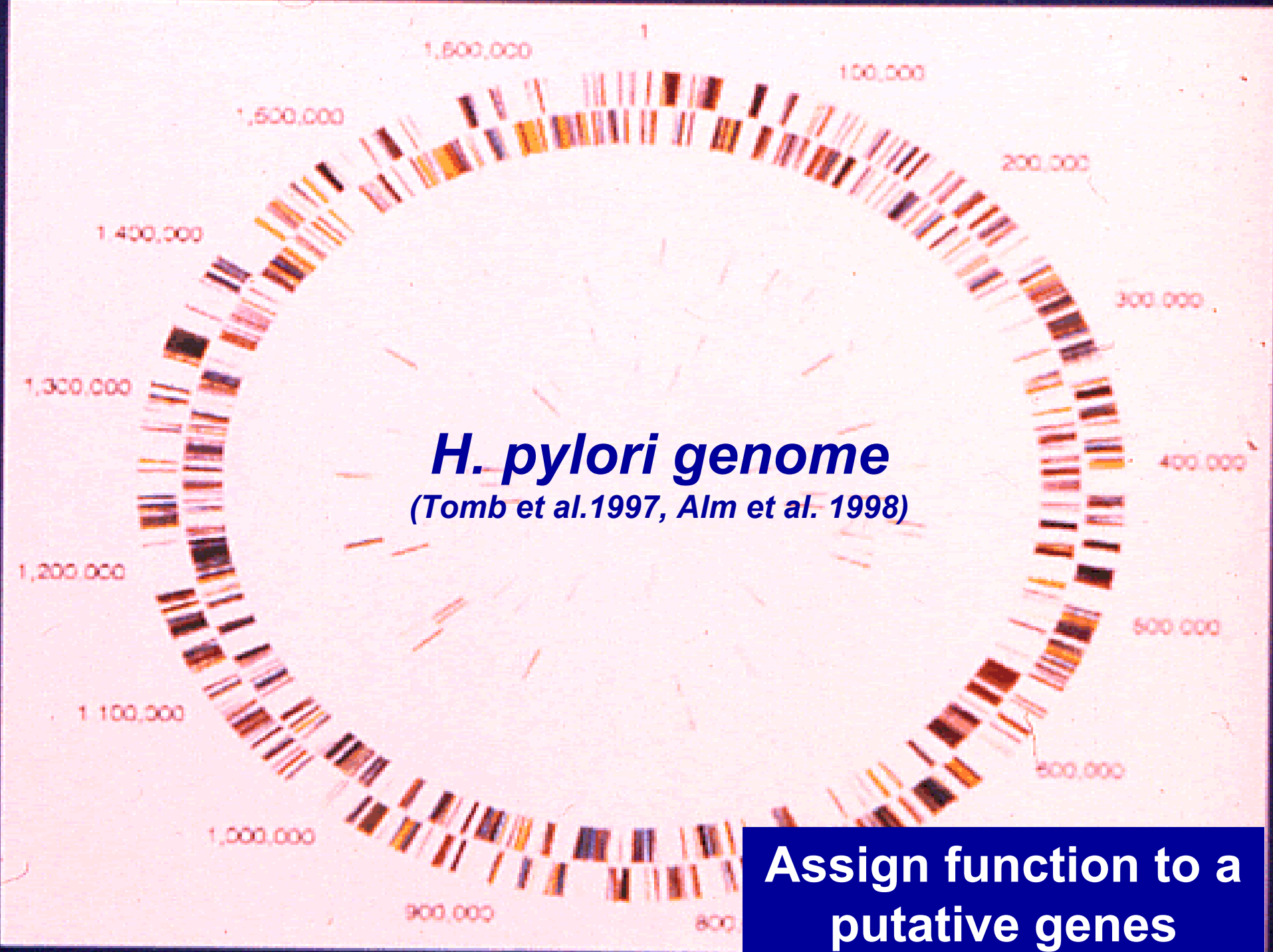


Censini et al., *PNAS 1996, 93: 14648-53*
Akopyants et al., *Mol Microbiol 1998, 28: 37-53*

Role of *cag* pathogenicity island and CagA protein



(*Covacci et Rappuoli, 2000*)

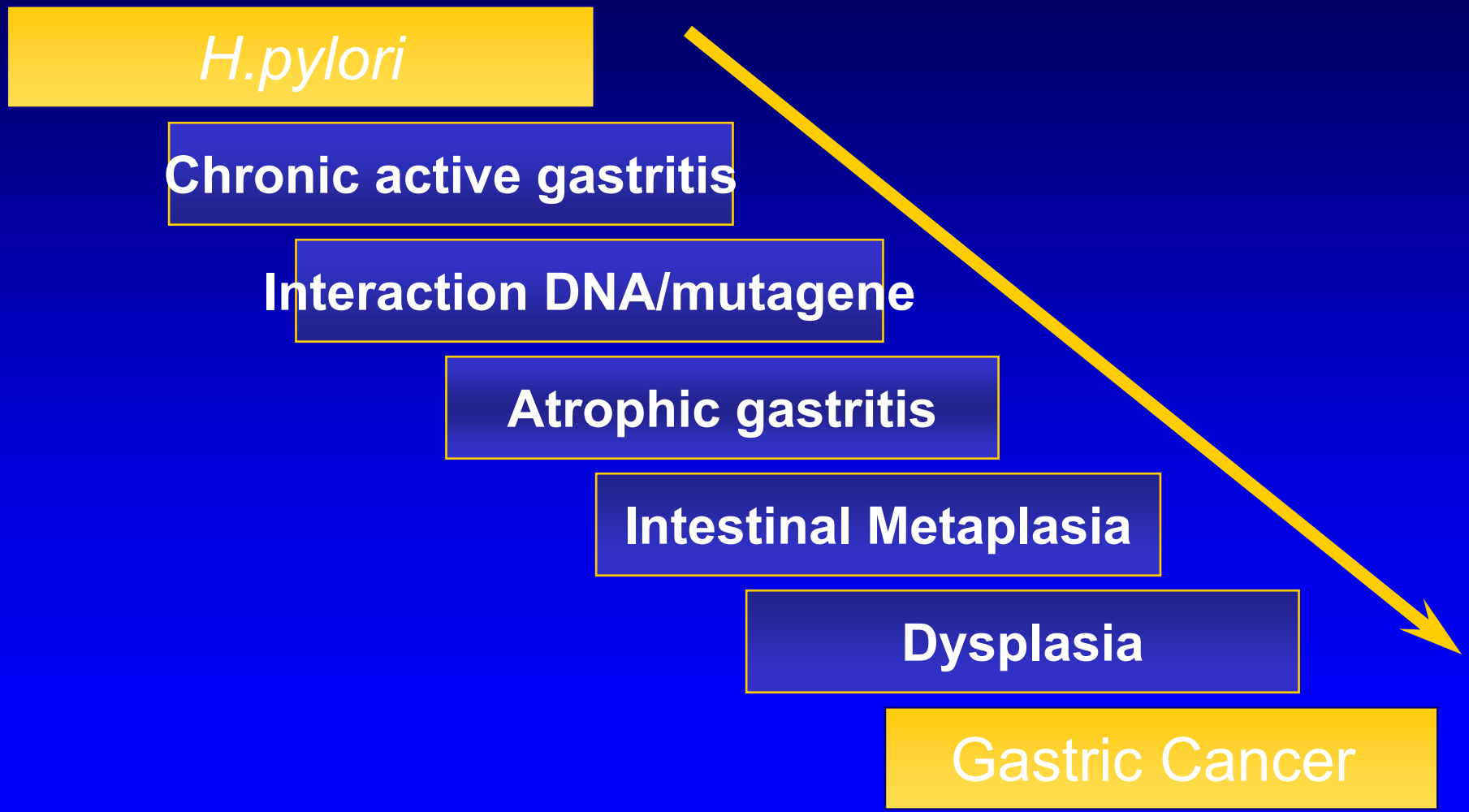


H. pylori genome

(Tomb et al.1997, Alm et al. 1998)

Assign function to a putative genes

H.pylori : from infection to gastric cancer



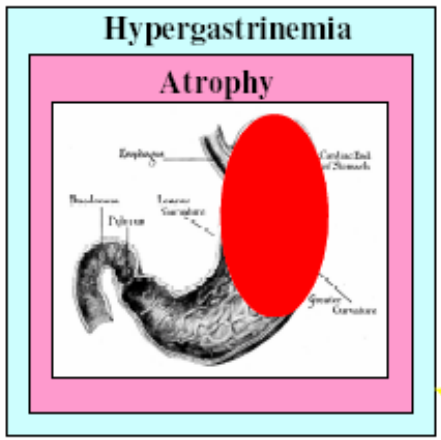
H. pylori and gastric pathology

Pro-inflammatory cytokines gene polymorphisms

***IL-1B*-511*T**
***IL-1-RN**2*2**
***IL-10* ATA haplotype**
***TNF*-A-308*A**
***IL-8*-251*A**

Innate immune response gene polymorphisms

***TLR4*+896*G**



Bacteria promoted factors

CagA, VacA
Endotoxins (LPS)
Growth factors, gastrins, COX-2-Prostaglandins
Reactive oxygen species

Environmental factors

Smoking
Dietary factors
Abuse of stimulants

H. pylori infection may be also responsible for gastric cancerogenesis that follows expression and action on gastric mucosa of cytotoxins (CagA and VacA), proinflammatory cytokines and gastrins.

June 1994

- Working group WHO/IARC

« Establishment of a definite link between H. pylori infection and gastric cancer in humans»

« H. pylori considered as *Group 1 (definite) carcinogen »*

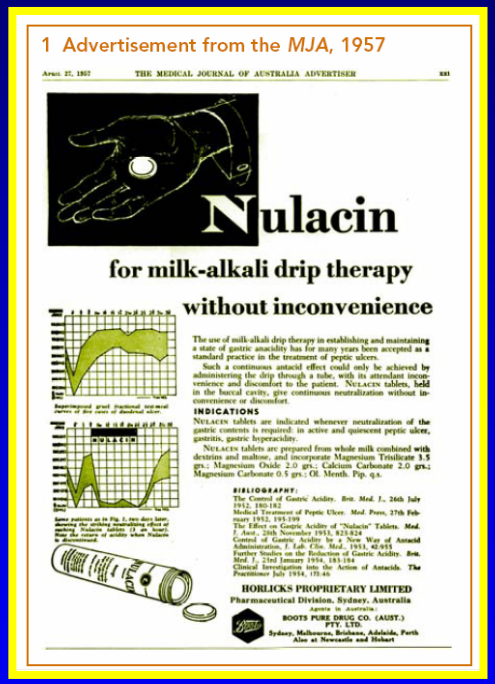
IARC Monograph. - Evaluation of carcinogenic risks for humans - 1994, Lyon, France

H.pylori and gastric cancer

- Epidemiologic data
  - Geographic and temporal concordance in incidence
- Cross-sectional case-control studies
  - 50 -100 % of gastric cancers infected with *Hp*
- Prospective nested case-control studies
  - *Hp* infection --> Risk of developing gastric cancer increased 2- to 9-fold
- Causal relationship in animal model
  - mongol gerbils

The only good *Helicobacter* is a dead *Helicobacter* !

Evolution of the understanding theories of gastroduodenal ulcers


1 Advertisement from the *MJA*, 1957

THE MEDICAL JOURNAL OF AUSTRALIA ADVERTISER

Nulacin

for milk-alkali drip therapy

without inconvenience

The use of milk-alkali drip therapy in establishing and maintaining a state of gastric anacidity has for many years been accepted as a standard practice in the treatment of peptic ulcers.

Such a continuous antacid effect could only be achieved by administering the drip through a tube, with its attendant inconvenience and discomfort to the patient. Nulacin tablets, held in the buccal cavity, give continuous neutralization without inconvenience or discomfort.

INDICATIONS

Nulacin tablets are indicated whenever neutralization of the gastric contents is required: in active and quiescent peptic ulcer, gastritis, gastric hyperacidity.

Nulacin tablets are prepared from whole milk combined with dextrins and maltose, and incorporate Magnesium Trisilicate 1.5 grs.; Magnesium Oxide 2.0 grs.; Calcium Carbonate 2.0 grs.; Magnesium Carbonate 0.5 grs.; Ol. Menth. Pip. q.s.

BIBLIOGRAPHY:

HORLICKS PROPRIETARY LIMITED
Pharmaceutical Division, Sydney, Australia

BOOTS PURE DRUG CO. (AUST.) PTY. LTD.
Sydney, Melbourne, Brisbane, Adelaide, Perth
Also at Newcastle and Hobart

50-60's

No acid… No ulcer

(K. Schwartz 1910)

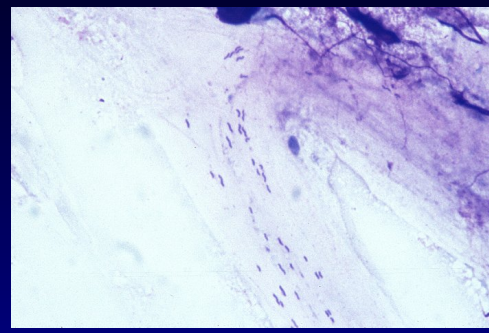
H2-receptor antagonists
(JW Black 1972)

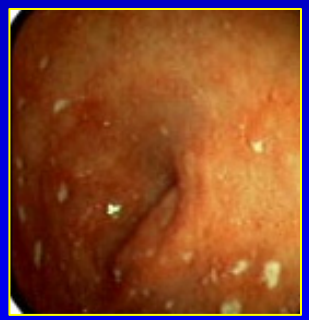
Proton-pump inhibitors
(G Sachs 1980)

No *Helicobacter*
…No ulcer

(B Marshall, 1988)

H. pylori infection:
Therapeutic indications





- Peptic ulcer disease* – active or not
- Gastric MALT lymphoma
- Atrophic gastritis
- Post-gastric cancer resection
- First degree relatives of gastric cancer patients
- Patient's wishes – after full consultation with their physician

Hp eradication Rx cost-effective*: > anti-H2 blockers for healing ulcers
= to maintainance Rx for preventing recurrences) (1-2 yrs)

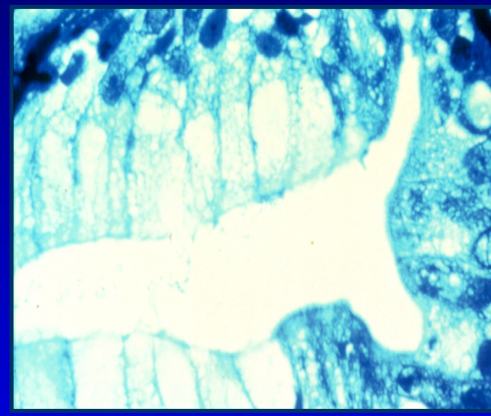
cochrane

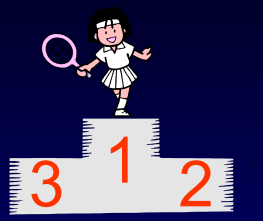
PubMed National Library of Medicine NLM

** Meta-analysis CRG, Ford, AJG 2004*

Characteristics of optimal drugs for the treatment of *H. pylori* infection

- High in vitro antibacterial activity
- High concentration in gastric mucosa/mucus
- Active by endoluminal and systemic routes
- Stable over wide range of pH (<2-7)
- Good tolerance / few side effects
- Low propensity for resistance
- Inexpensive





First choice antibiotics

Clarithromycin

- Most active single agent
- Good diffusion in gastric mucosa
- Synergy with acid-suppressive agents

- Increasing resistance
- Decreased efficacy in areas of high resistance (20%)
- High cost

Amoxicillin

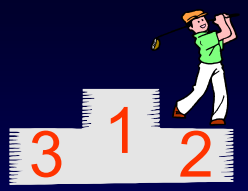
- No resistance development
- Activity not affected by acidic pH

- Broad-spectrum
- Side effects (gastrointestinal, dermatologic)

Metronidazole

- Activity not influenced by pH
- Clinical efficacy little affected by resistance
- Inexpensive

- Side effects (gastrointestinal)
- High resistance rates in several areas (>40%)



Second choice antibiotics

Tetracyclines

- Resistance rare
- Synergy with bismuth salts
- Clinical efficacy in quadruple therapy
- Inexpensive

- Side effects (gastrointestinal, rash)

Fluoroquinolones

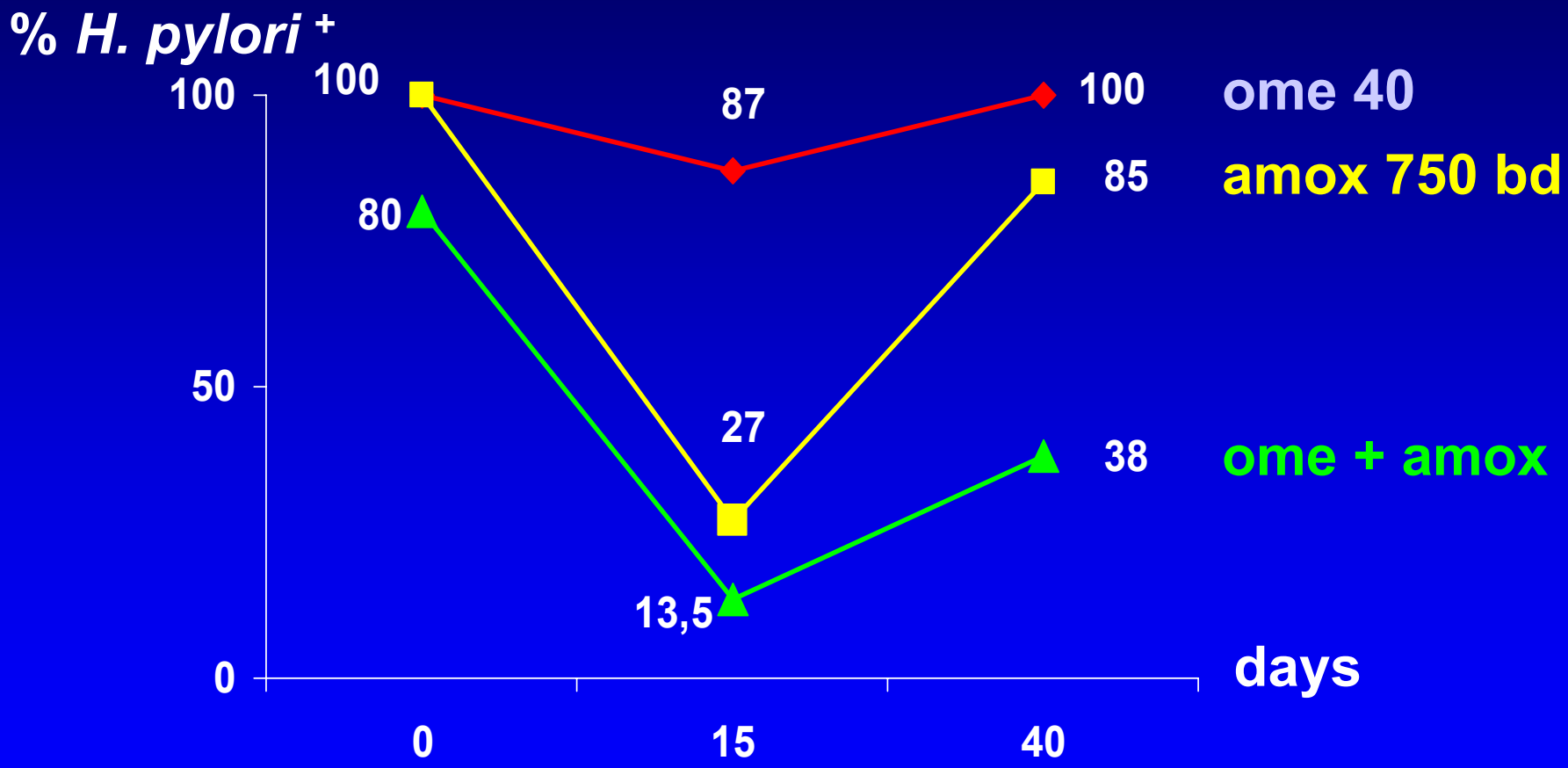
- Potential interest for rescue therapy if previous treatment failures
- Well tolerated

- Wide usage of FQ in other indications
- Poorly active at acidic pH
- Resistance increasing

Proton-pump inhibitors

- Bacteriostatic activity against *H. pylori*
- Synergy with some antimicrobials (metro, clari, tetra)
- Effect of acid suppression (↑ gastric pH):
  - increases activity of several antibiotics (macrolides, quinolones)
  - Improved penetration of bismuth salts in gastric mucosa
  - Increased concentrations of metro, clari, tetra in the stomach
- Improve clinical efficacy of dual antimicrobial regimens

Adjuvant effect of Omeprazole on *H. pylori* eradication by amoxicillin



Unge, Bordeaux 1988

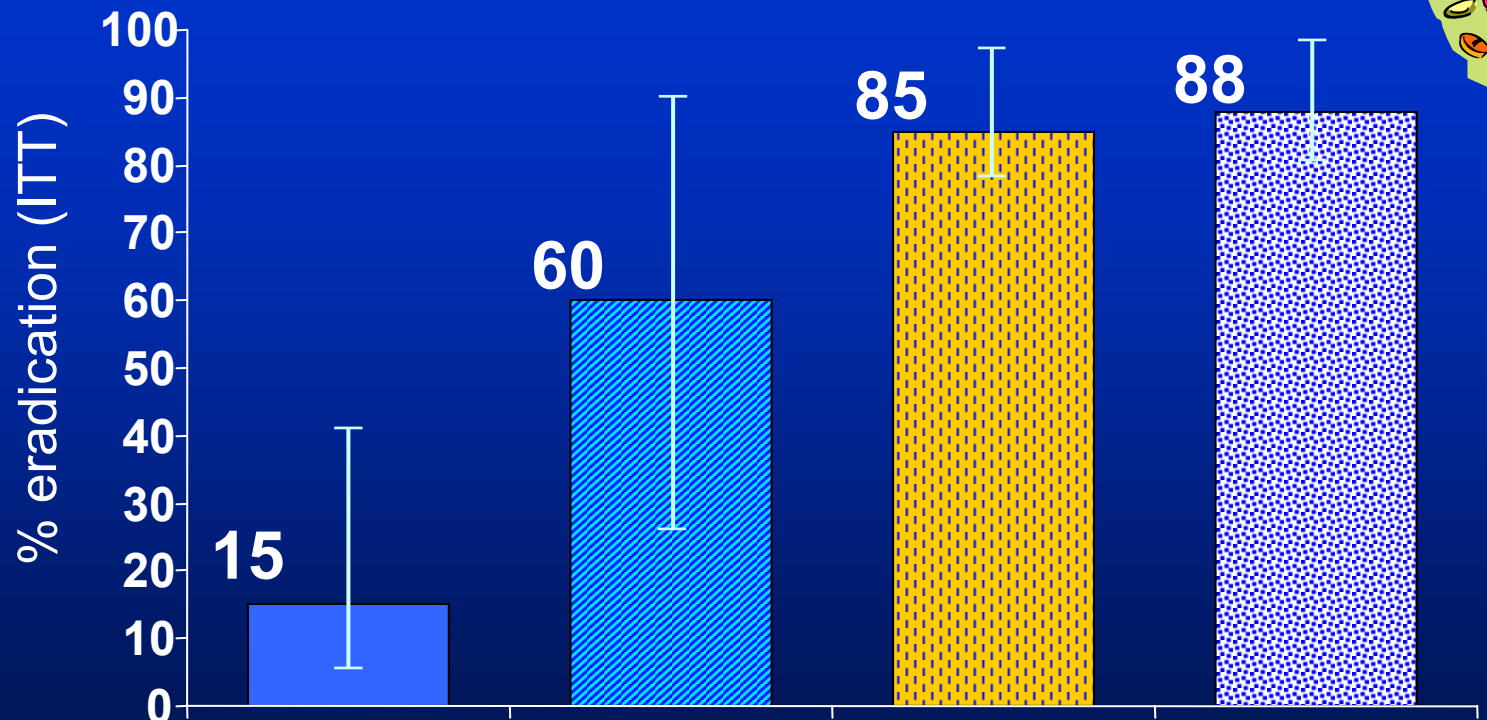
Therapeutic principles (1)

Rational choice of therapeutic agents

- Based on clinical experience
- Association regimens including :
  - amoxicillin
  - clarithromycin
  - 5-Nitroimidazoles
  - Tetracycline
  - Bismuth salts
- Other agents active in vitro have proven ineffective in vivo

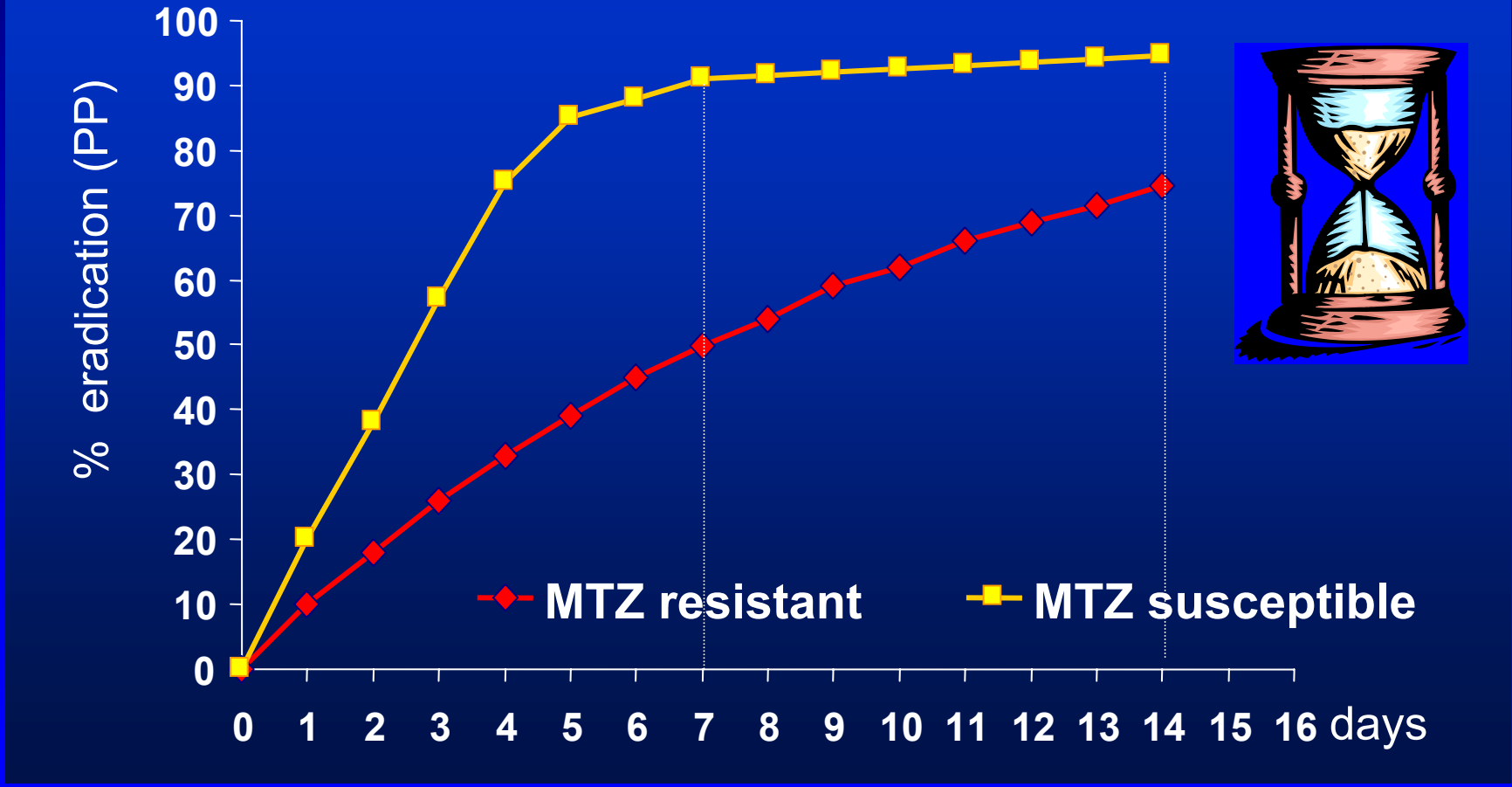
Hp : Therapeutic Principles (2)

➔ Importance of drug associations



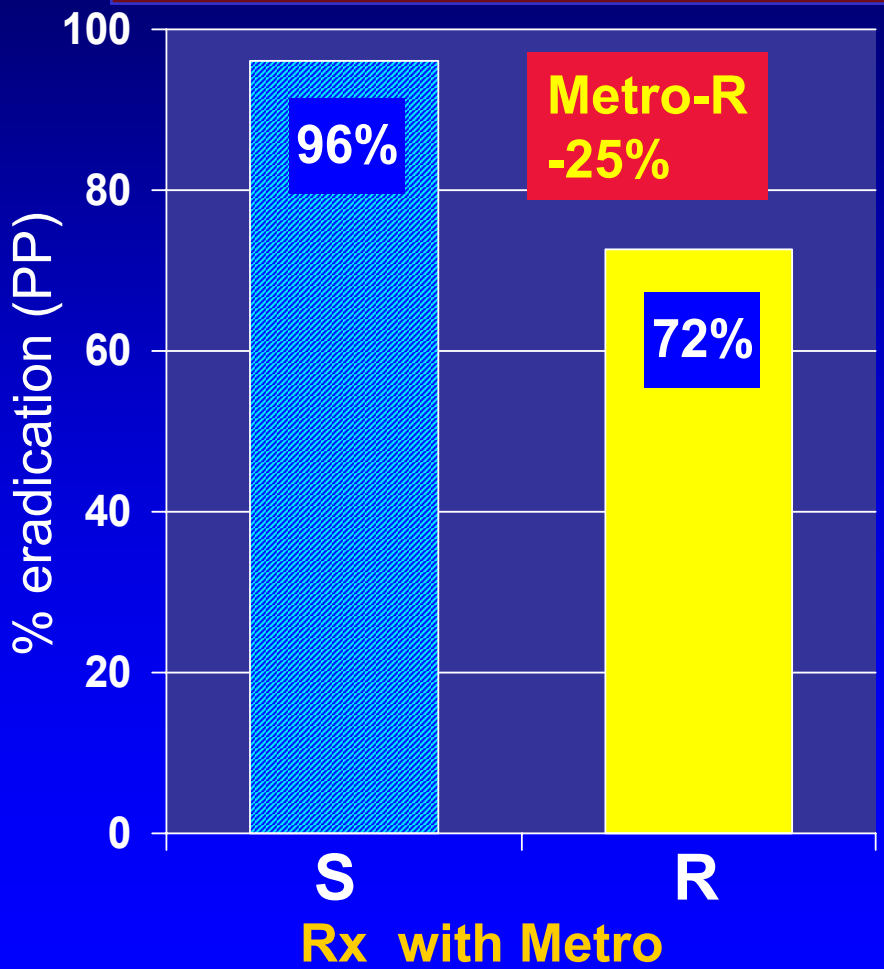
Hp : Therapeutic principles (3)

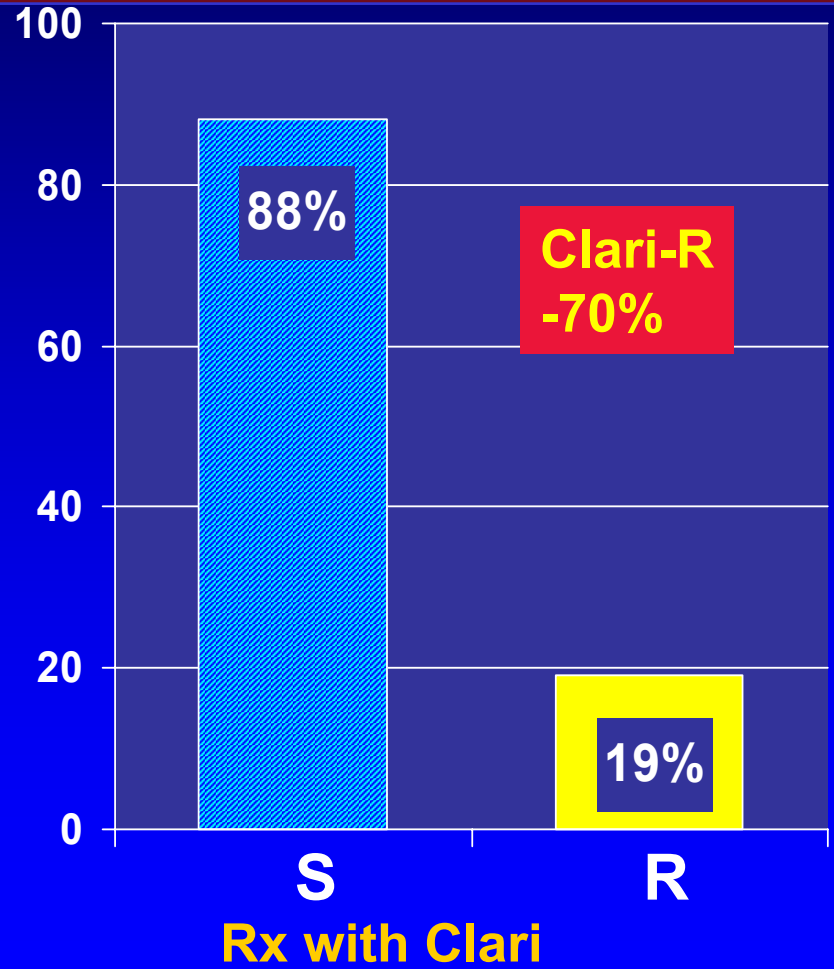
Treatment duration: eradication rates with bismuth based triple therapies



Hp : Therapeutic principles (4)

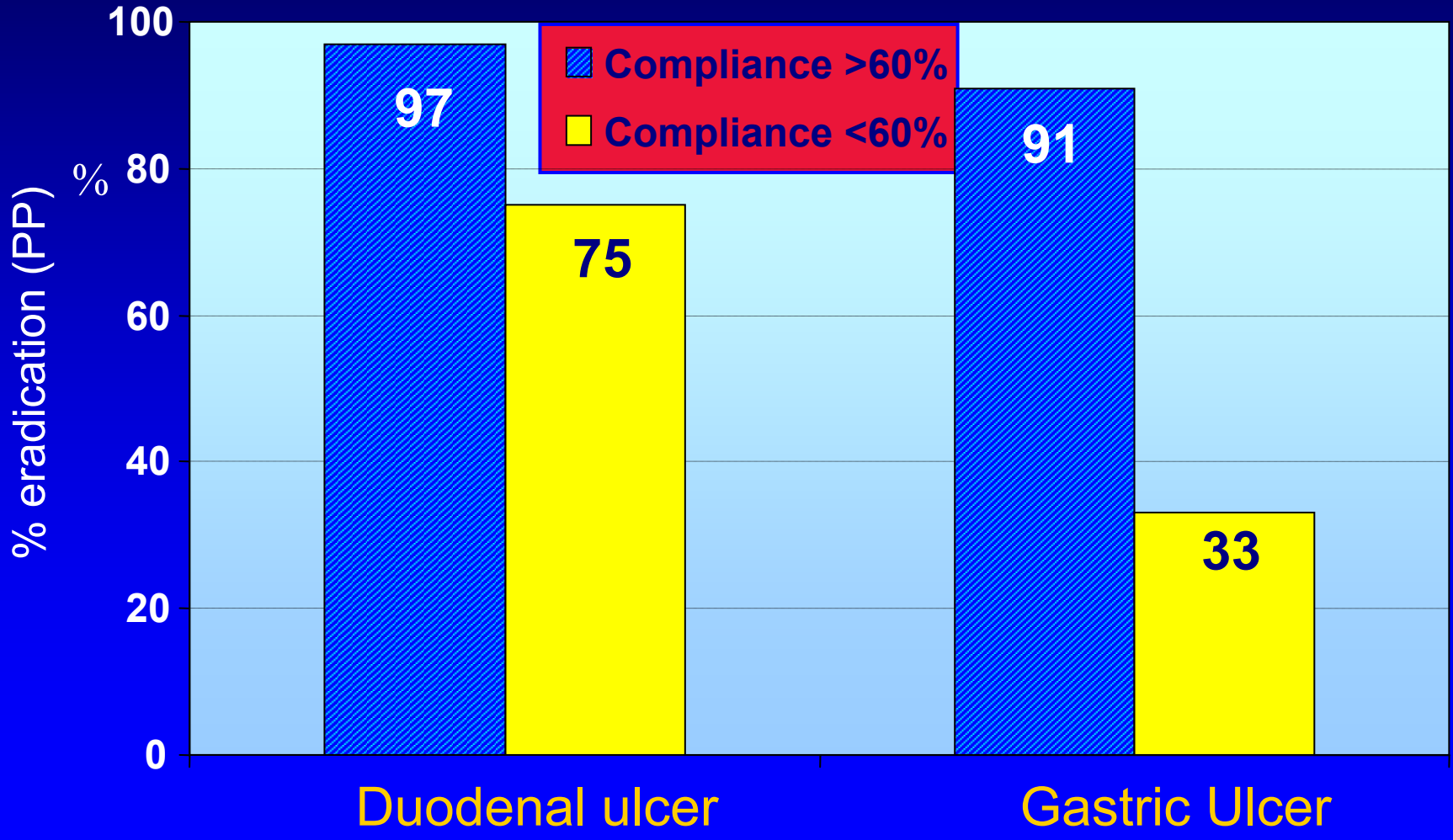
➔ **Importance of resistance on Hp eradication**





Mégraud, Gut 2004

Importance of patients compliance for eradication of *H. pylori*



Graham, Gastroenterol 1992

Factors of importance for predicting outcome of therapy in Hp infection

PROVEN

- Choice of agents
- Choice of associations
- Compliance
- Antimicrobial resistance
- Dosage
- Duration of therapy

POSSIBLE

- Drug formulation
- Number of daily administration
- Drug intake in relation to meal
- Polymorphism of CYP2C19 in metabolism of PPIs

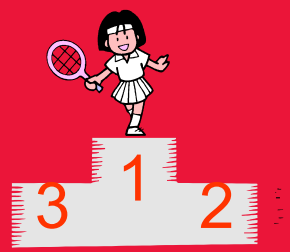
EUROPEAN HELICOBACTER STUDY GROUP

First line therapy

Maastricht 2005-3 consensus report

1 week, Triple therapy, 2x/daily
(PPI + 2 ABs)

PPI standard dose bid (omeprazole, lanso, panto)
+ Clarithromycin 500 mg bid (C)
+ { Amoxicillin 1000 mg bid (A)
  or
  Metronidazole 500 mg bid (M) }

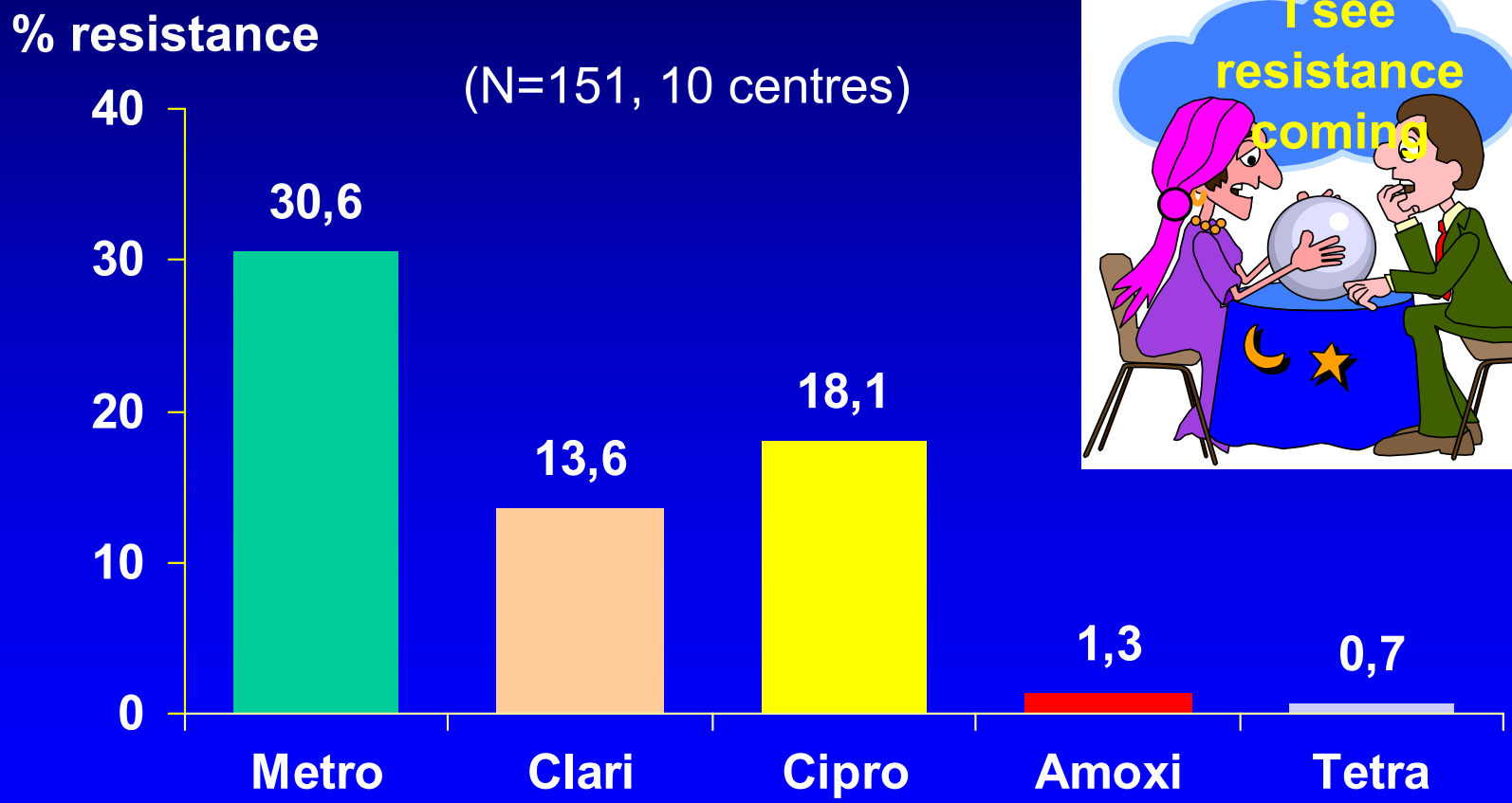


CA preferred in area with Metro-R > 40%

≥ 80% cure rates on ITT to basis

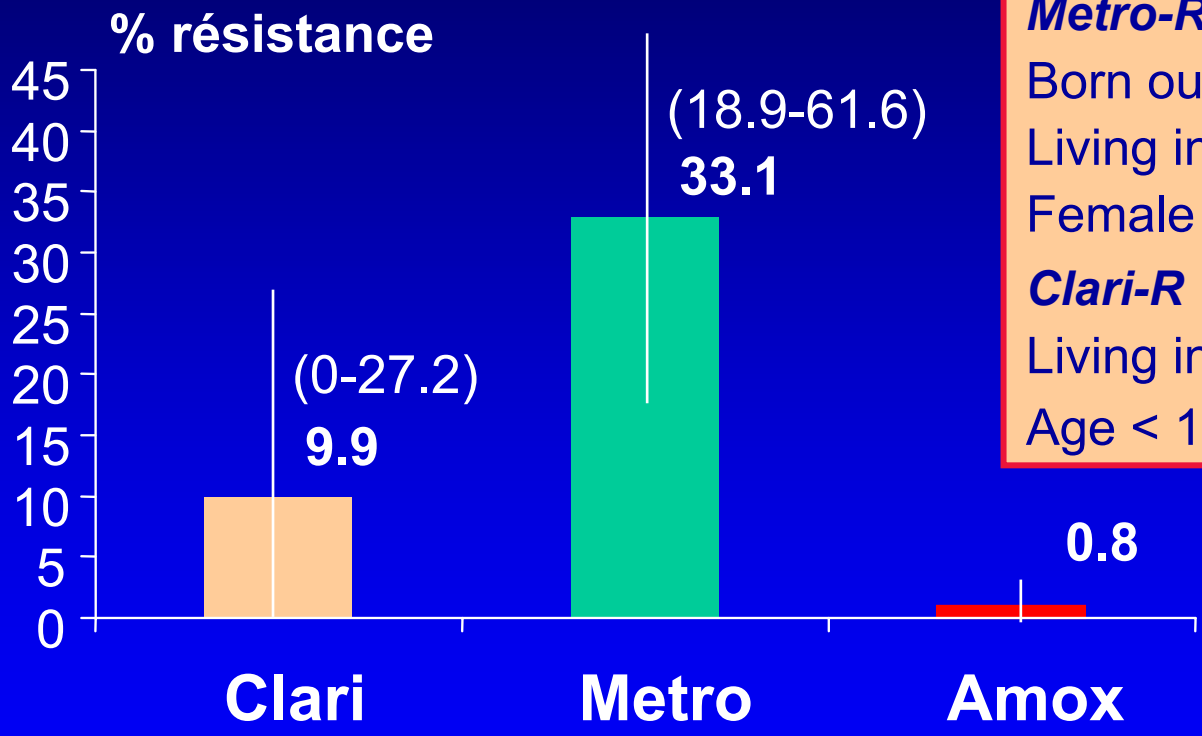
Malfertheiner, APT 2002

Primary resistance rates of *H. pylori* in Belgium



Belgian Helicobacter Study Group, 2004

Antimicrobial resistance *of H. pylori* in Europe



Risk factors for resistance

Metro-R

Born outside Europe (OR: 2.7)

Living in East. Europe (OR: 1.9)

Female (OR: 2.3)

Clari-R

Living in South. Europe (OR: 2.3)

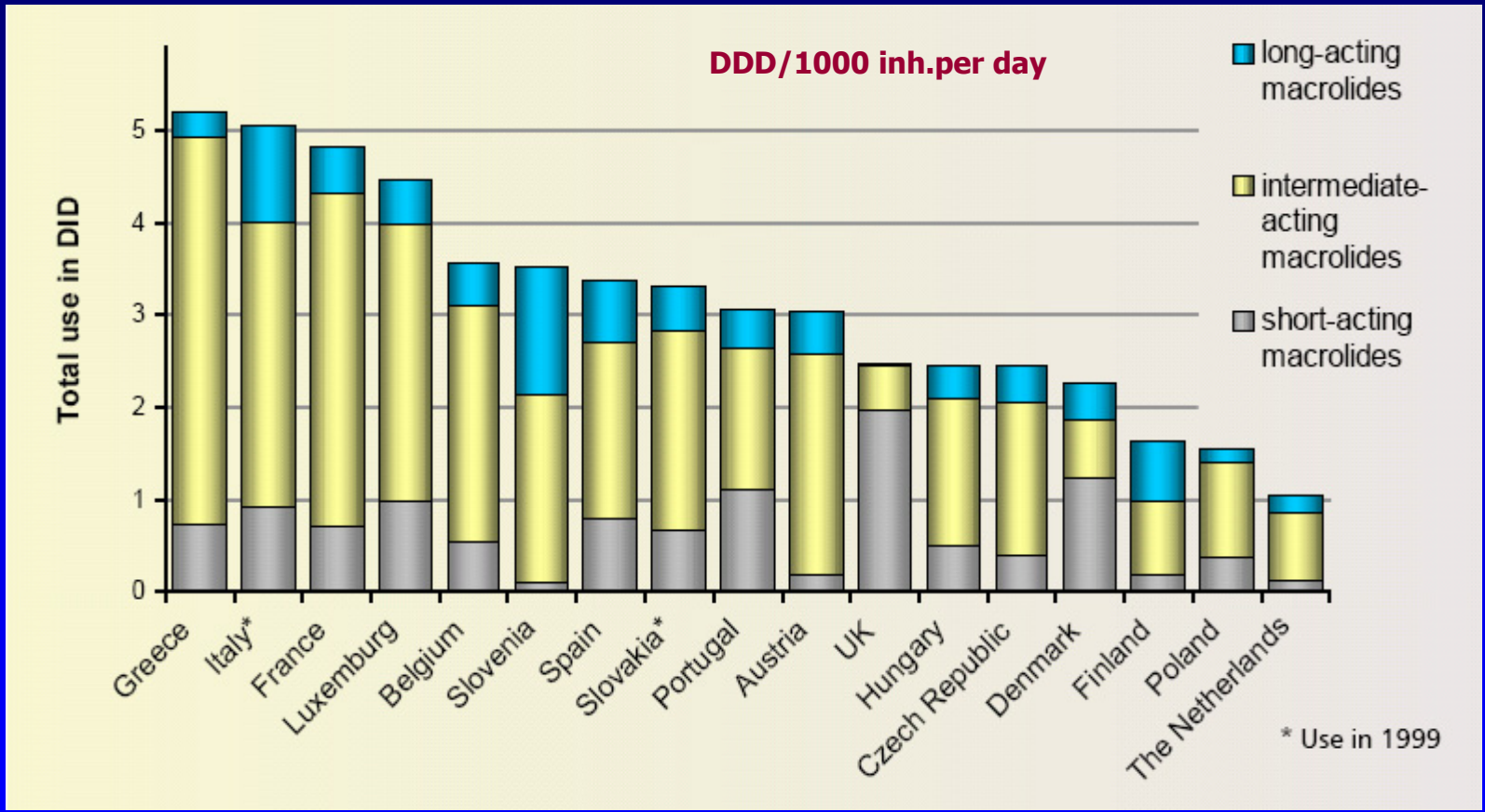
Age < 12 yrs (OR: 1.8)

22 centres, 17 pays

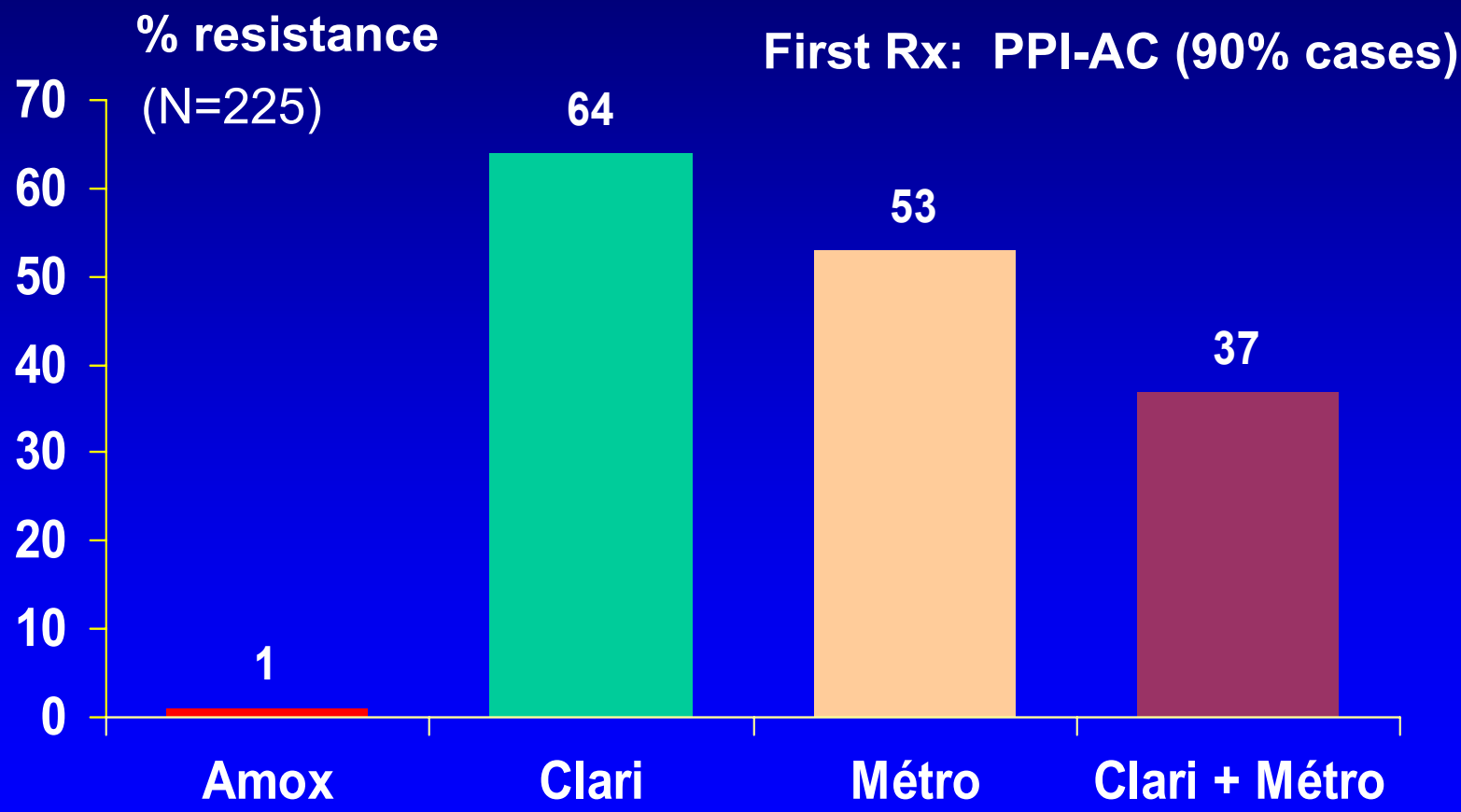
N=1274 (N° d'isolats/Centre = 64, valeurs extrêmes: 21-115)

Glupczynski , EJCMID 2001

Consumption of macrolides in 17 European countries in 1998



Secondary resistance of *H. pylori* after eradication failure



Mégraud et al. Gut 2001

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Second line therapy

Maastricht 2005-3 consensus report

Bismuth quadruple therapy, 10-14 d

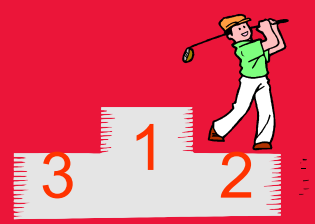
(failure, allergy, intolerance, high level of resistance to clari)

PPI standard dose bid (omeprazole, lanso, panto)

+ Bismuth salt (CBS) 120 mg qid

+ Metronidazole 500 mg tid

+ Tetracycline 500 mg qid
  or
  Amoxicillin 500 mg tid



Unaffected by Clari-R; little influenced by Metro-R

Cost < to PPI-triple therapy

≥ 85% cure rates on ITT to basis

Fishbach, APT 2004

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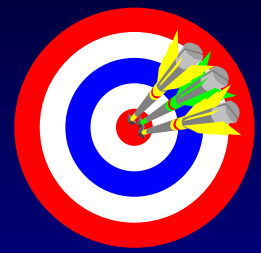
Rescue therapy



after failure of two courses of different therapies

Treatment adjusted on the basis of culture and susceptibility results (antibiogramme)

Should be handled on « Case-by-case » basis

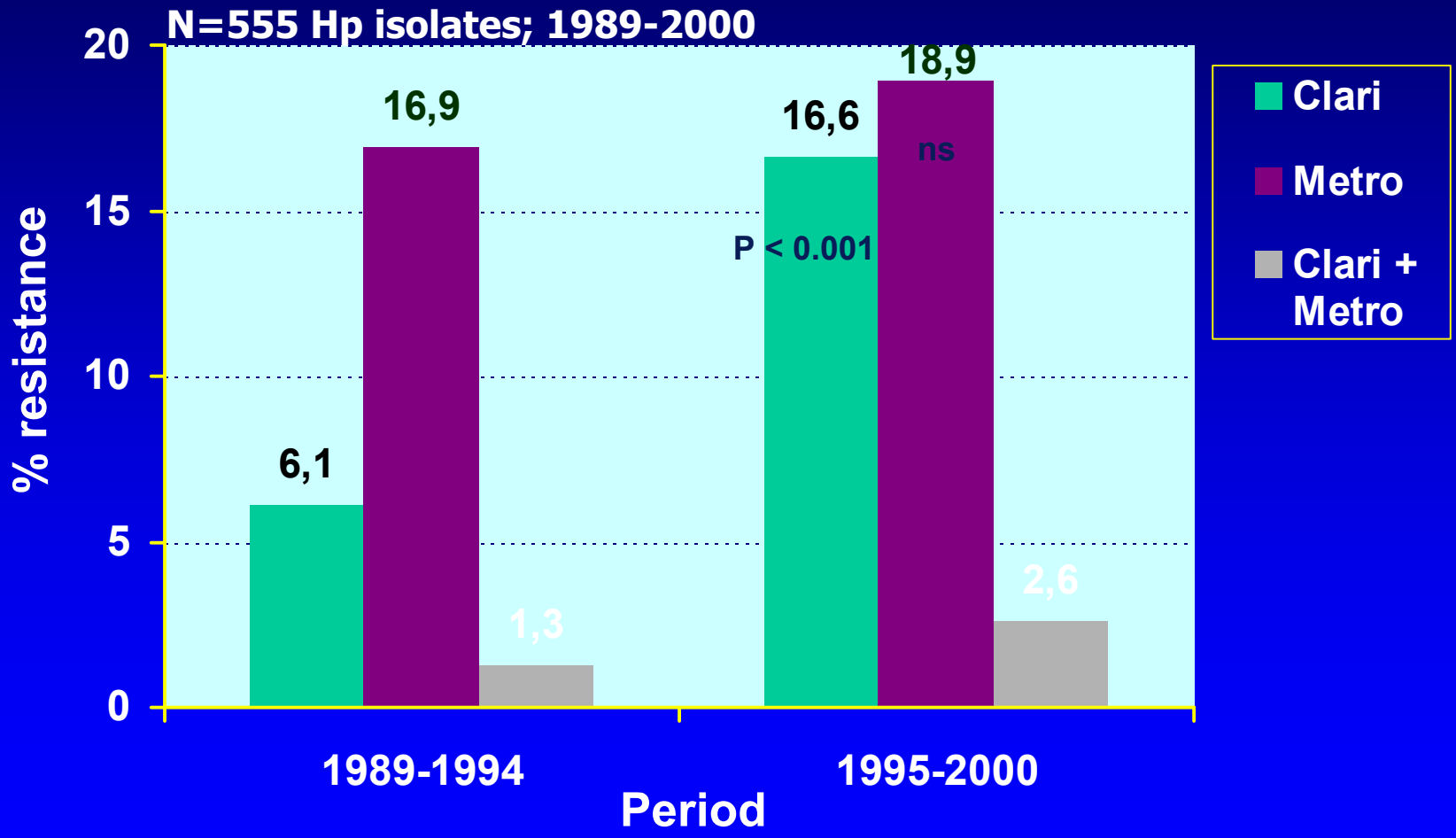
Avoid re-using same agents (clari, metro)

Proposed triple therapies (under evaluation):

PPI + Amox + fluoroquinolones (Levoflo, Moxiflo)
rifamycins (rifaximin, rifabutin)

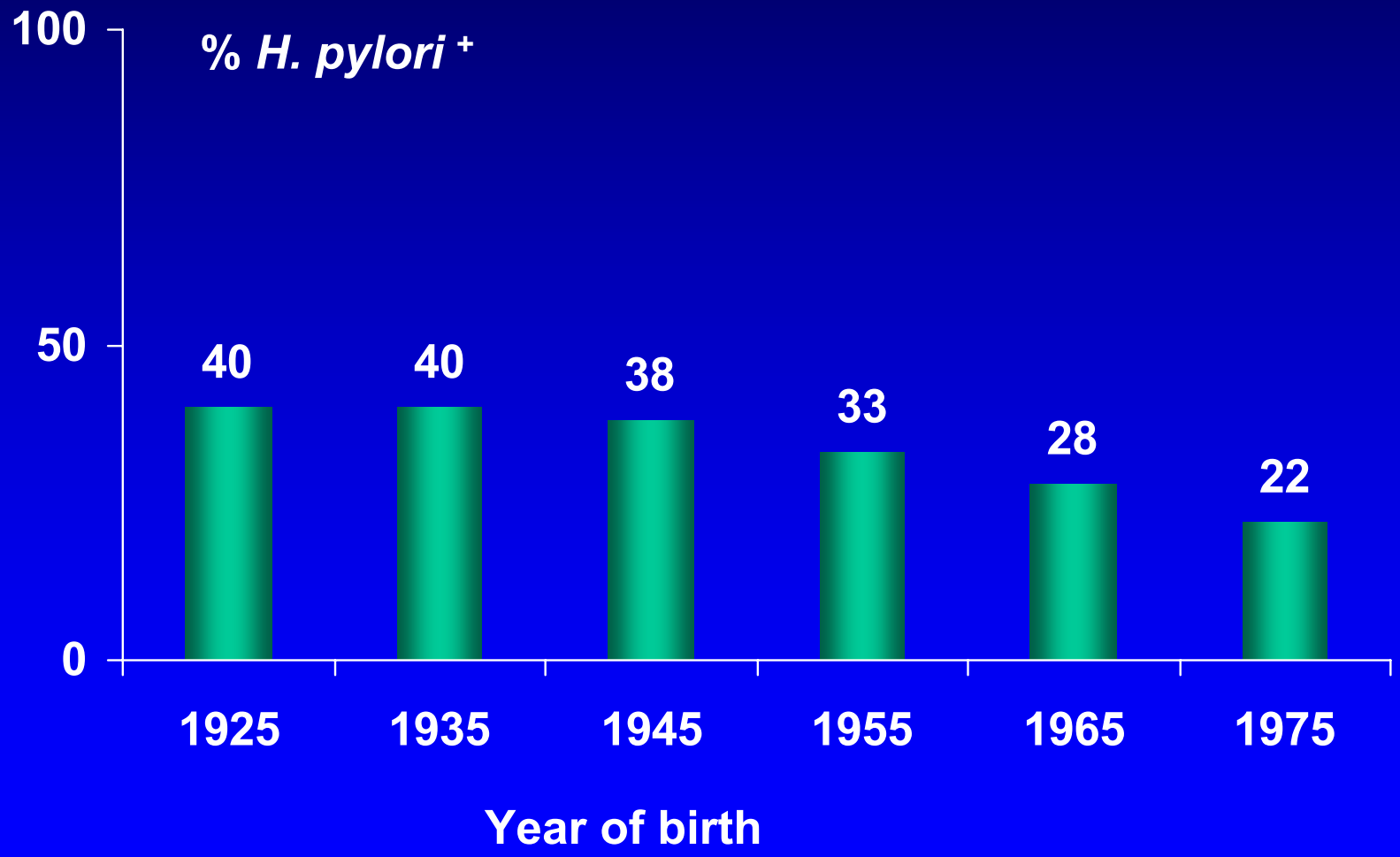
APUA
ALLIANCE FOR THE PRUDENT USE OF ANTIBIOTICS

Evolution of *H. pylori* resistance



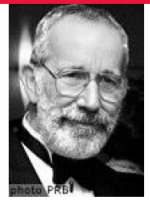
Bontems et al., PIJD 2001

Evolution of prevalence of *H.pylori* infection in Europe



Perspectives

- ***H. pylori*** **infection becoming more rare**
- **Consequences of infection in terms of burden of associated diseases (ulcer, cancer) is going to be with us for a while**



Barry J. Marshall

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born 1951

J. Robin Warren

Australia

Perth, Australia

born 1937

The *H. pylori* saga ending in a Nobel Prize ….

- …Being at the right place at the right time, and seeing what other people had seen but thinking what nobody else thought…
- The role of chance…
- … »Challenging established dogmas with passion, tenacity and prepared mind …
- Possibility of making important discoveries as clinicians in the course of daily practice…