Antibiotics promote colonization by resistant bacteria and development of resistance during therapy

Elisabeth Nagy

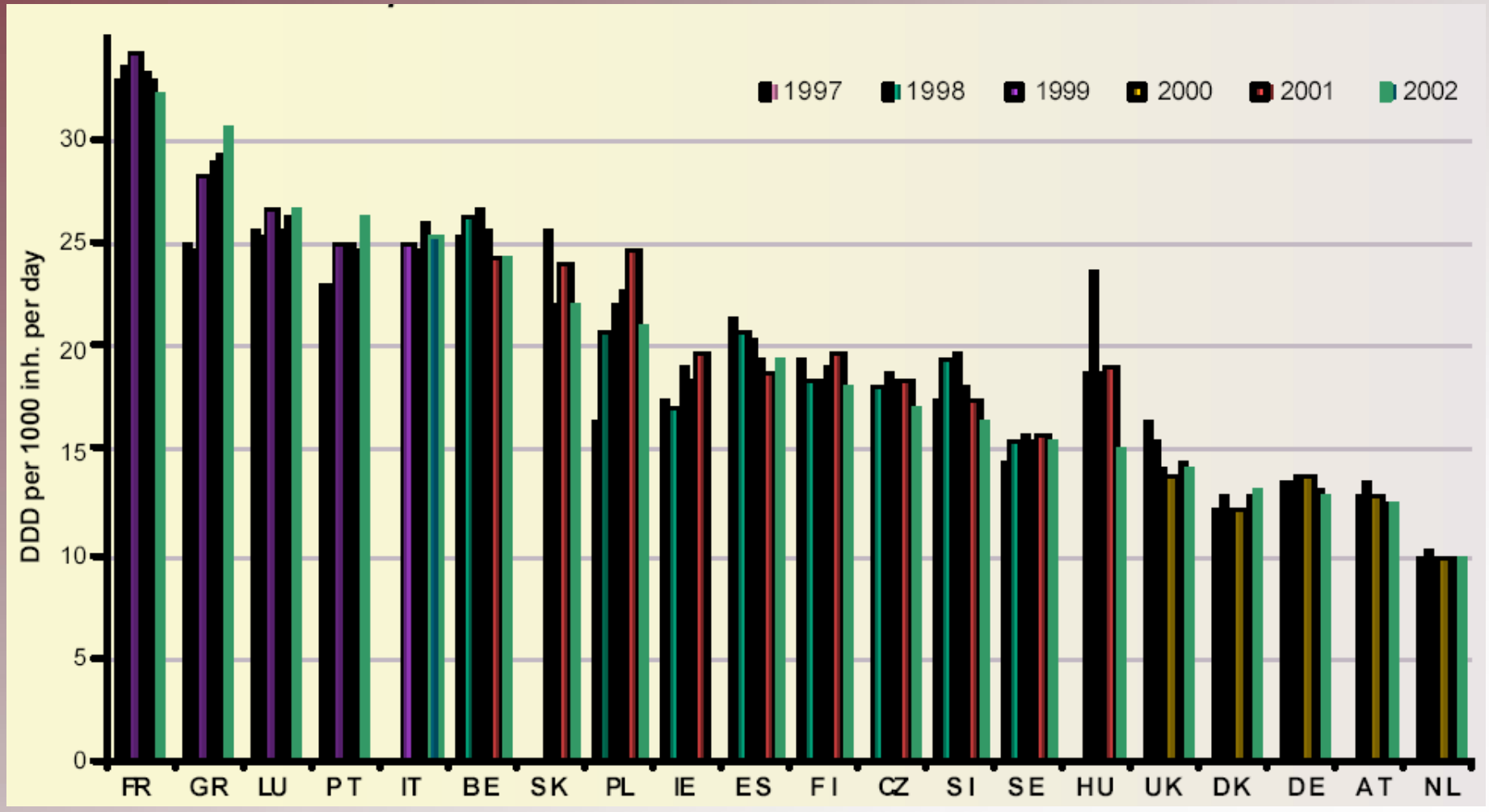
Institute of Clinical Microbiology, Faculty of Medicine, University of Szeged

4th ESCMID School, Szeged, Hungary (2005)

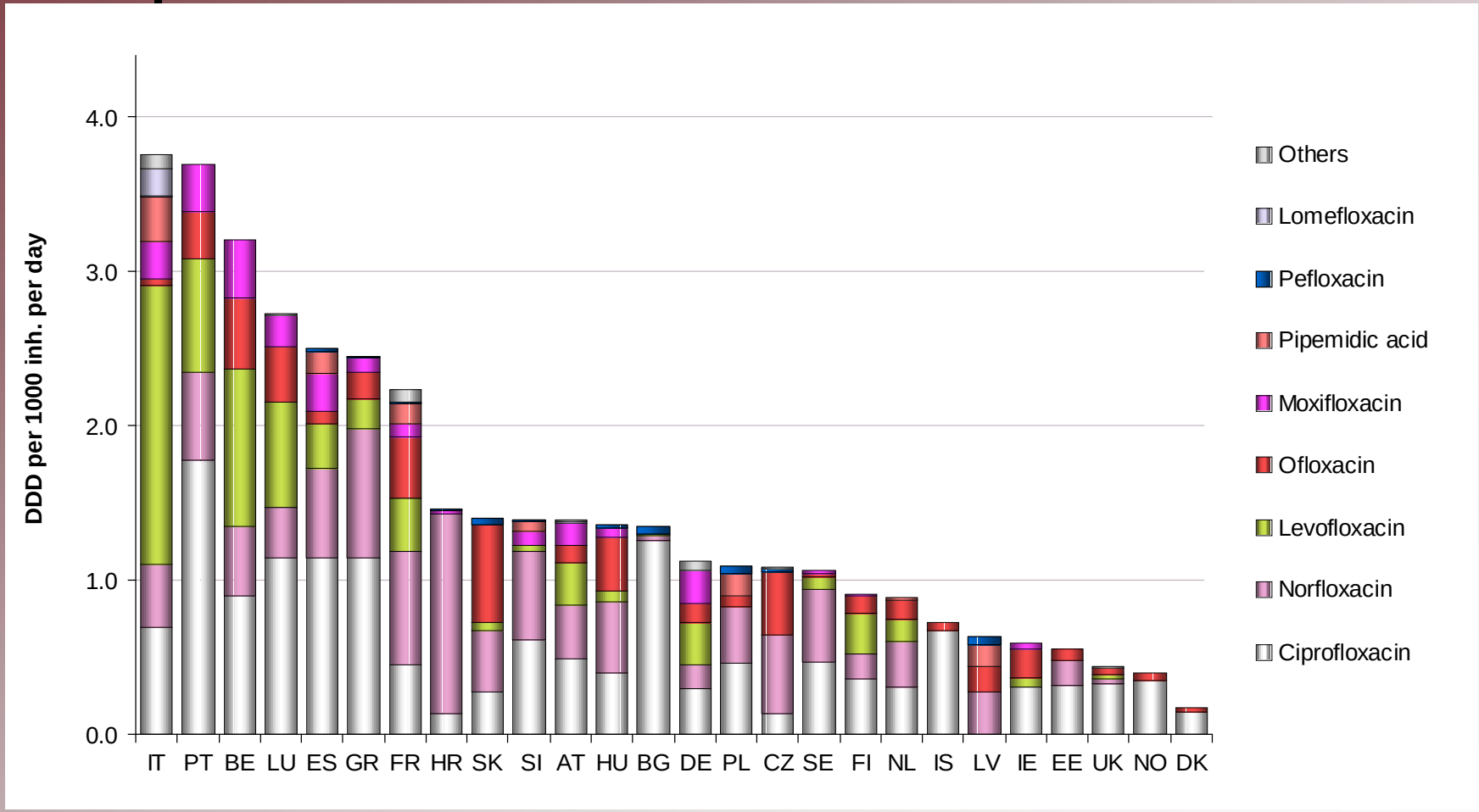
Antibiotic usage worldwide

- ~100 million antibiotic prescriptions in the USA/year
- The antibiotic market worldwide consumes around ~$2x10^5$ tonnes of different antibiotics
  - ~50% used in agriculture
  - ~50% used in humans
- More and more broad-spectrum antibiotics are developed
- Selection of antibiotic-resistant bacteria is inevitable

Trend of antibiotic consumption in ambulatory care in 20 European countries: 1997-2002 (ESAC)



Outpatient use of quinolones in 26 European countries in 2002



Outpatient Antibiotic Use in Europe and Association with Resistance

Herman Goossens, M.D., Ph.D., Matus Ferech, Pharm.D., Robert Vander Stichele, M.D., Monique Elseviers, Ph.Dand the ESAC Project Group.

Lancet 2005; 365: 579-87

Constitution of normal, indigenous flora of humans

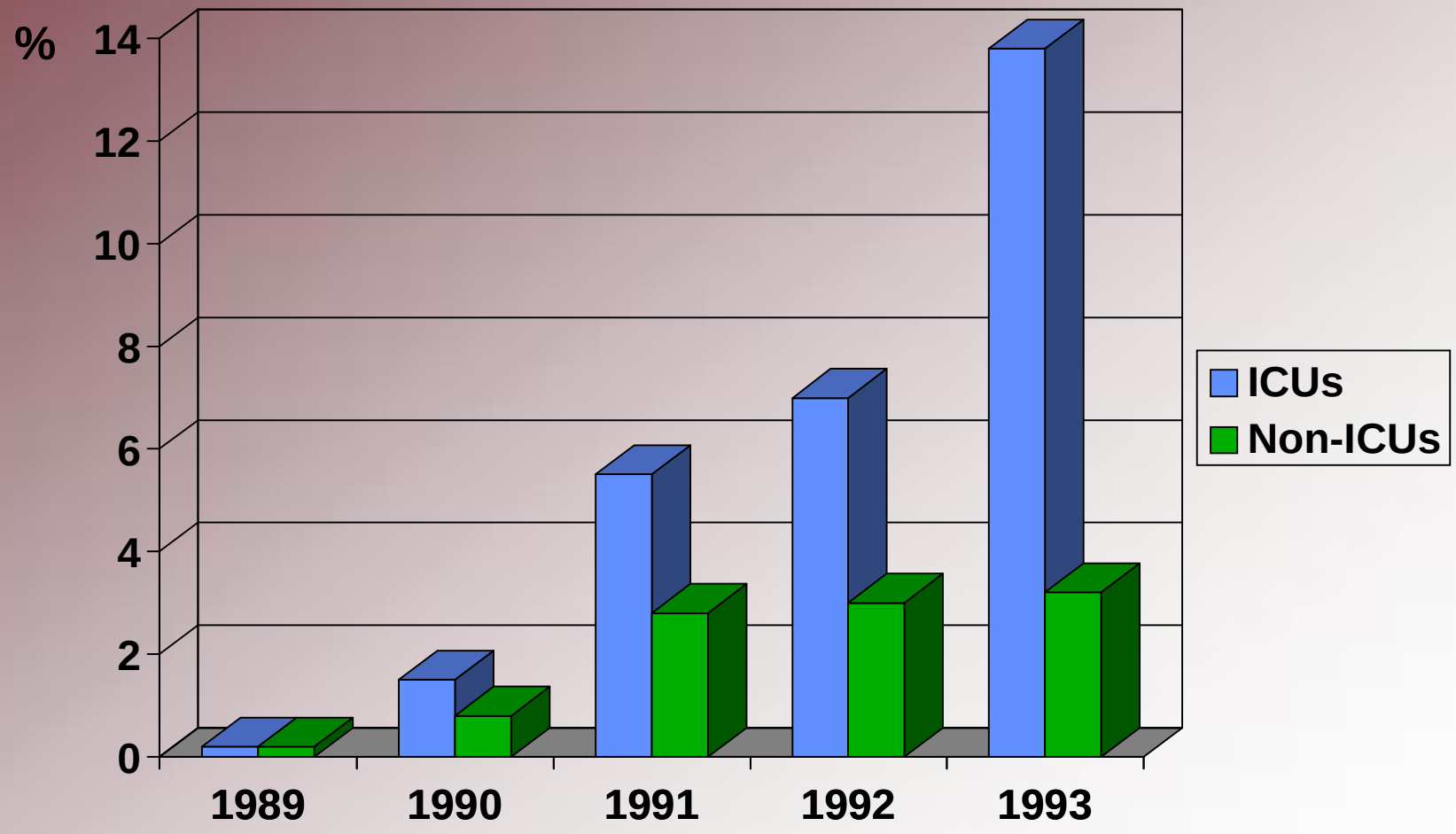
	aerobe/anaerobe
Oral flora	1/100-1 000
Gastrointestinal flora	1/1 000-10 000
Vaginal flora	?
Skin flora	100/1

Antibiotics used for any reason may select resistance in the population

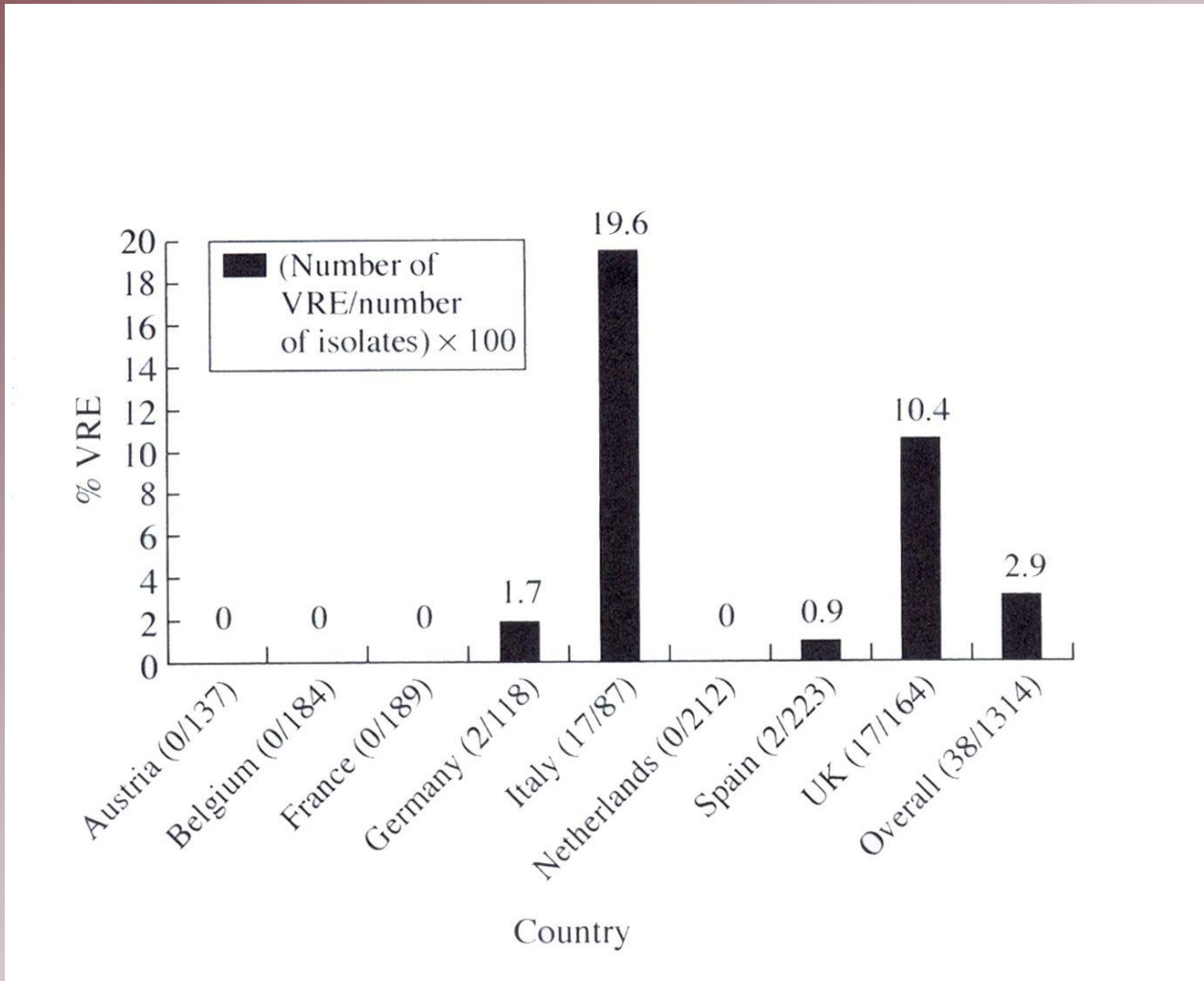
Possible consequences of antibiotic usage concerning the normal flora

- Disturbance of the composition and the number of normal flora ⟶ overgrowth of pathogenic bacteria, fungi
- Usage / over usage of an antibiotic (vancomycin) ⟶ selection of VRE in the faecal flora
- Less frequently proven, but it happens in the case of anaerobic bacteria as well

Percentage of vancomycin resistant enterococci in different US hospitals

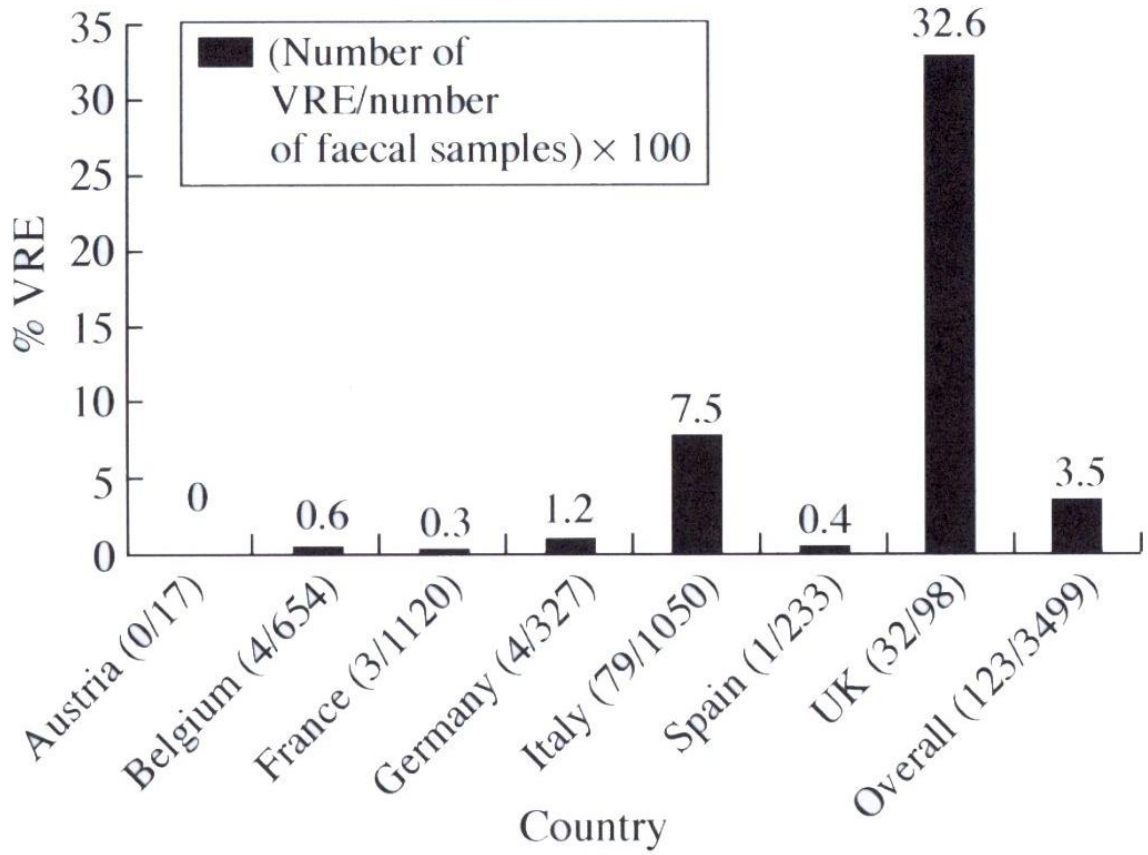


H. Goossesns et al.: European survey of vancomycin-resistant enterococci (JAC 2003)



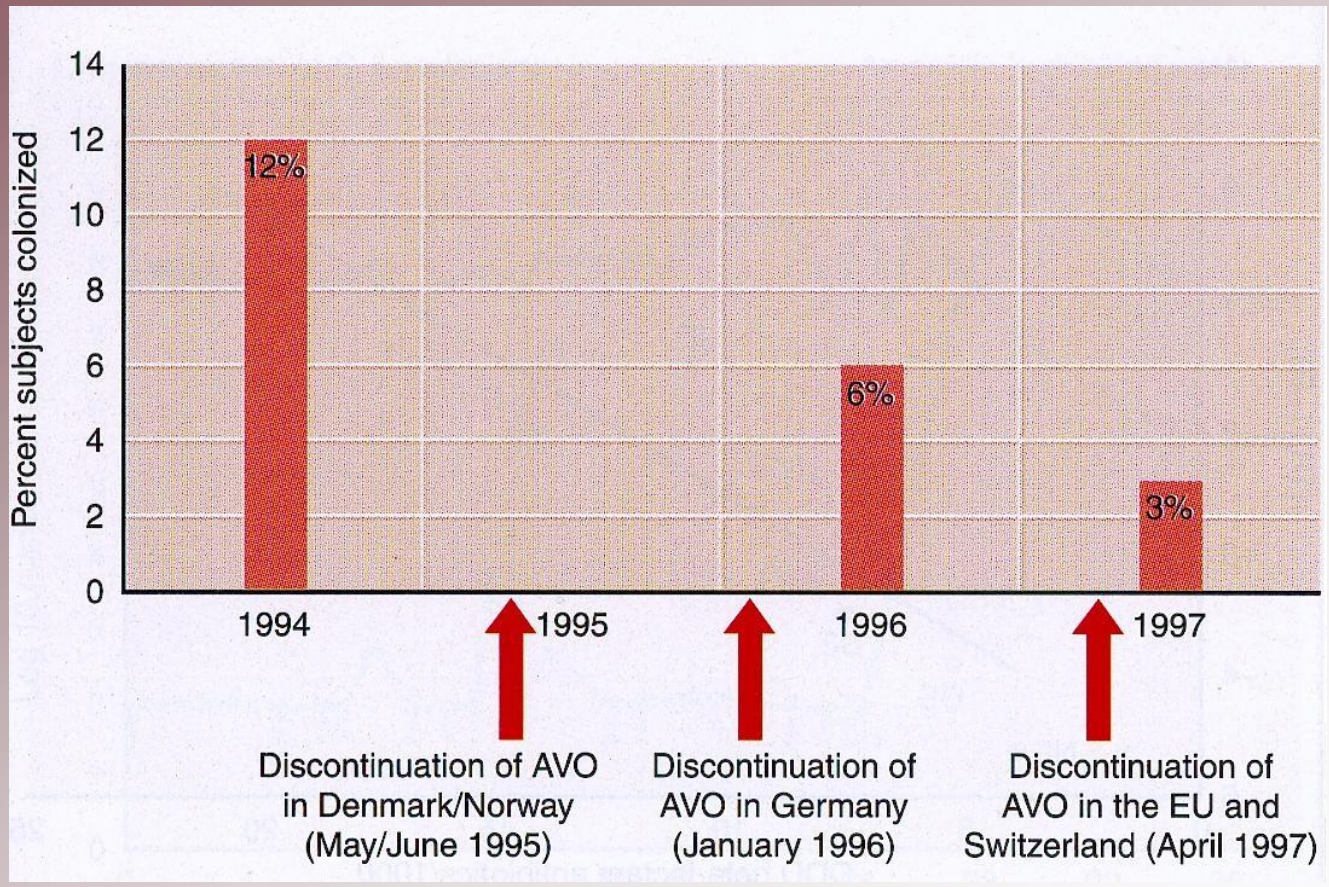
Rate of vanA and vanB types among 1314 clinical enterococcal isolates

H. Goossesns et al.: European survey of vancomycin-resistant enterococci (JAC 2003)

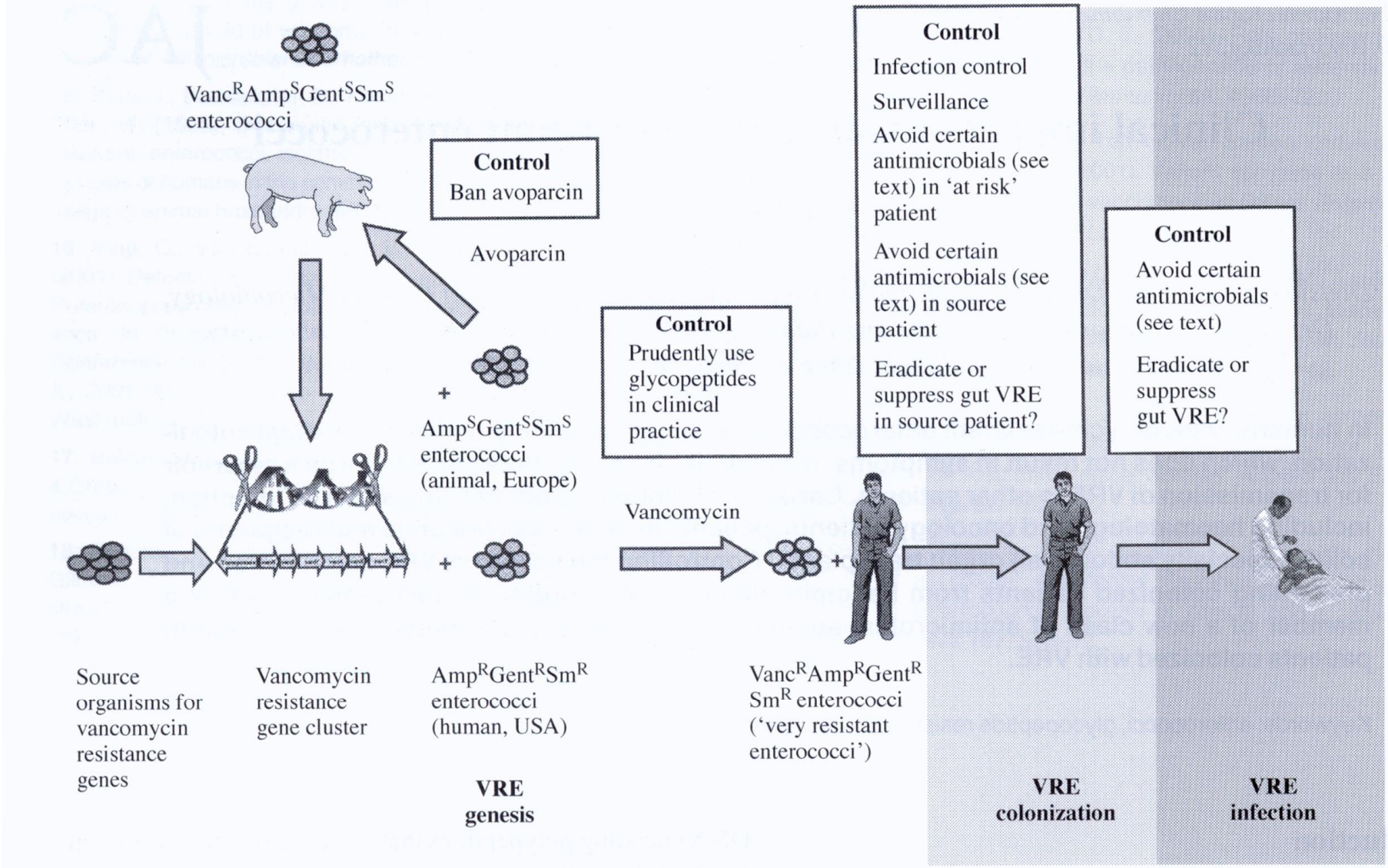


Rate of vanA and vanB enterococcal types among 3499 faecal samples

Relationship between antibiotic use in the husbandry and decrease of faecal colonization with glycopeptide resistant enterococci in the community



Andremont A. ASM News 2003 December



R. Patel: Clinical impact of vancomycin-resistant enterococci JAC 2003

Selection of antibiotic resistance in the normal flora

Impact of treatment of *H. pylori* on the normal gastrointestinal microflora. (Adamsson I. et al.: CMI 6: 175-177; 2000)

- Half of the world's population is infected with *H. pylori*. A considerable proportion of the infected population have chronic gastritis, peptic ulcer or gastric carcinoma.

- The triple therapy (OAM/OCM), however, affects not only the *H. pylori,* but the normal flora too.

OAM: omeprazole, amoxicillin, metronidazole

OCM: omeprazole, clarithromycin, metronidazole

14 patients received 20 mg omeprazol + 1000 mg amoxicillin + 400 mg metronidazole orally twice daily for 7 days

- 741 normal flora (oral, gastric and intestinal) isolates were tested for amoxycillin susceptibility before and after treatment

Isolates	Changes in Amo. resistance
E. coli	MIC increased
Streptococcus spp	MIC increased
Staphylococcus spp	30% → 64%
Enterococcus spp	MIC increased
Enterobacteriaceae	12% → 76%
Bacteroides spp	no changes, all resistant

16 patients received 20 mg omeprazol + 250 mg clarithromycin + 400 mg metronidazole orally twice daily for 7 days

- 742 normal flora (oral, gastric and intestinal) isolates were tested for clarithromycin sensitivity before and after treatment

Isolates	Changes in clarithromycin MIC resistance	
Streptococcus spp	↑	5% → 74%
Enterococcus spp	↑	2% → 92%
Enterobacteriaceae	↑	
Bacteroides spp	↑	2% → 76%

Metronidazole MIC50/MIC90 for *Bacteroides* spp: 0.5/0.5 → 0.25/2 mg/l

Faecal colonisation by carbapenem-resistant Bacteroides *in healthy persons*

Faecal samples of healthy volunteers were cultured anaerobically in BHI broth containing 8 or 64 µg/ml meropenem for 48 h.

No. of	Szeged (Hungary)		Nottingham (U.K.)	
Faeces tested	**145**		**79**	
MER res *Bacteroides* (MIC: ≥8 µg/ml)	**4**	*B. fragilis (cfiA+)* *B. capillosus (cfiA-)* *B. distasonis (cfiA-)* *B. vulgatus (cfiA-)*	**3**	*B. fragilis (cfiA+)* *B. fragilis (cfiA-)* *B. fragilis (cfiA-)*

Spec. meropenemase: 4.7-99 nmol/min/mg protein

Sóki, Edwards et al. (Int. J. Antib. Agents 2004)

Faecal colonisation by carbapenem-resistant Bacteroides *in healthy persons*

- **Prevalence of *cfi*A gene in *B. fragilis* isolates**

In healthy volunteers	**2 /224**	**(0.89%)**
In clinical cases*	25/1284	(1.94%)

- **Prevalence of ≥32 µg/ml carbapenem resistance with IS elements**

In healthy volunteers	**0/224**	**(0%)**
In clinical cases*	4/1284	(0.31%)

*ESGARAB study 1999-2000

Development of resistance in the normal flora during antibiotic usage

Tetracyclin was given to chickens in low doses during 12 weeks to improve meat production

Isolation from faeces	Tetracyclin given	Tetracyclin not given
E. coli Tet^r (Amp^r) from chickens	+++	-
E. coli Tet^r (Amp^r) from humans	+++	-

S.B. Levy (Boston, US)

Development of resistance during therapy

(Tzouvelekis L.S. et al.: J. Med Microbiol. 1994)

- A 67-year-old patient, who had leukemia, was admitted with symptoms of sepsis and fever (39 ^{o}C). Empirical therapy: ceftazidime + netilmicin. From the blood taken before antibiotic therapy *E. aerogenes* (E1) was cultured (ceftazidime:S, netilmicin:S)

- On day 13, he had a new fever peak and *E. aerogenes* was cultured from his blood again (E2), which proved resistant to all beta-lactam antibiotics except imipenem. The therapy was changed to imipenem.

Cont.

- On day 19, his blood culture was again positive with a slow-growing *E. aerogenes* (E3). The isolate was resistant to imipenem, and intermediate resistant to fluoroquinolones.

- Isolates E1, E2 and E3 gave the same pattern on the use of PFGE.

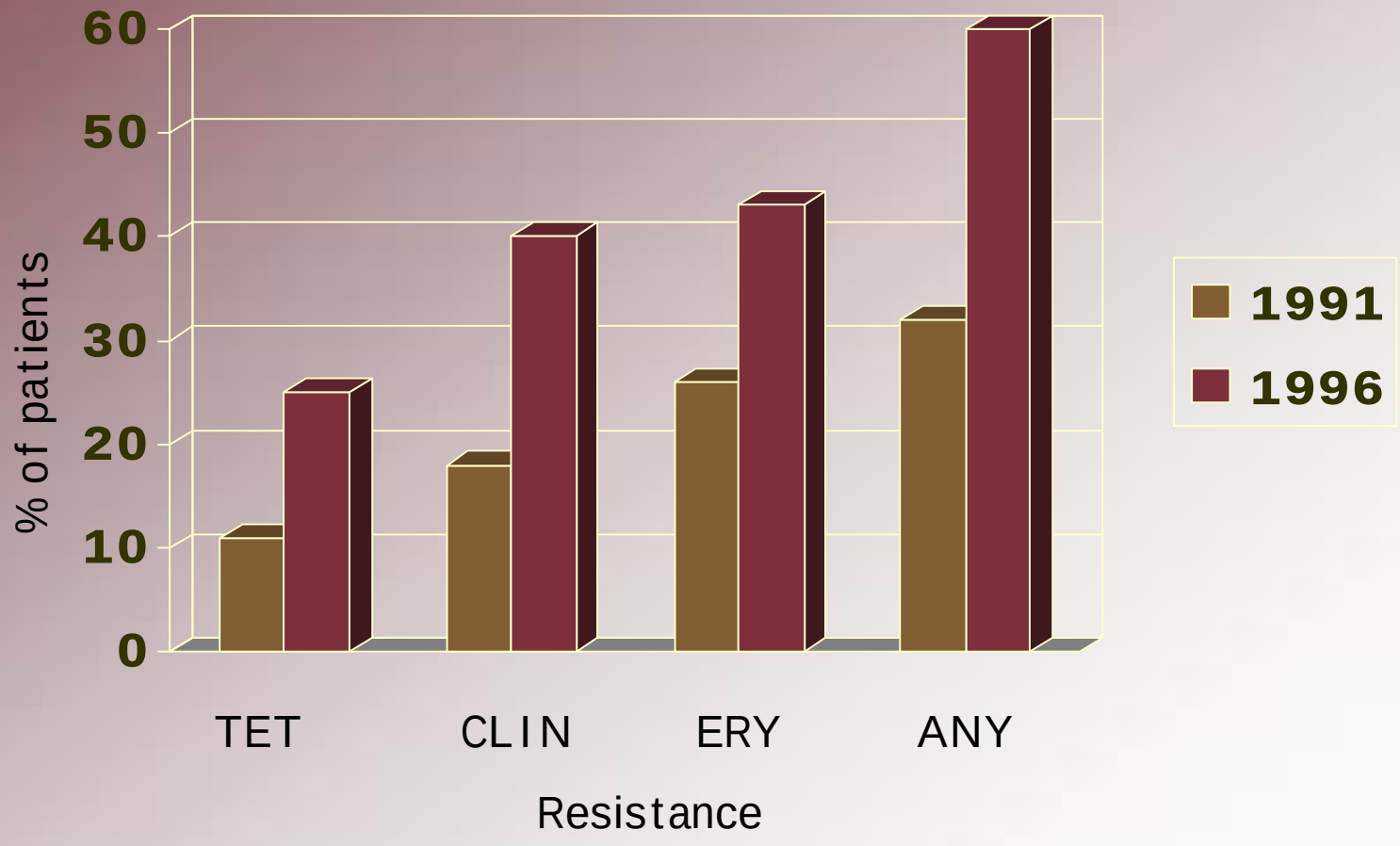
Cont.

- It was possible to prove that
  - isolate E1 had an inducible beta-lactamase
  - during therapy, a stably derepressed mutant was selected from isolate E2 by using cephalosporin
  - in isolate E3, OMP profile analysis proved that a 39 kDa protein (porin) was missing, probably again due to mutation

Trends in imipenem resistance
(resistance breakpoint ≥ 16 mg/L)

- 1985: Introduction of imipenem in the USA and Europe
- 1988-1989 first European *Bacteroides* resistance study
  - no resistant strain was found
  - 4 (0.3%) of 1289 *B. fragilis* strains had an MIC >4 µg/ml
- 1996: Introduction of meropenem in the USA
- 1999-2000 second European *Bacteroides* resistance study (ESGARAB)
  - 0.8% of the strains were resistant (8 *B. fragilis*, 2 others)
  - 16 (1.2%) of 1 284 *B. fragilis* group strains had an MIC >4 µg/ml (only 86% *B. fragilis*)
- 2001-2002: Introduction of ertapenem in the USA and Europe
- ???? Next European study ????

Selection of antibiotic-resistant P. acnes *in acne patients (skin carriage)*



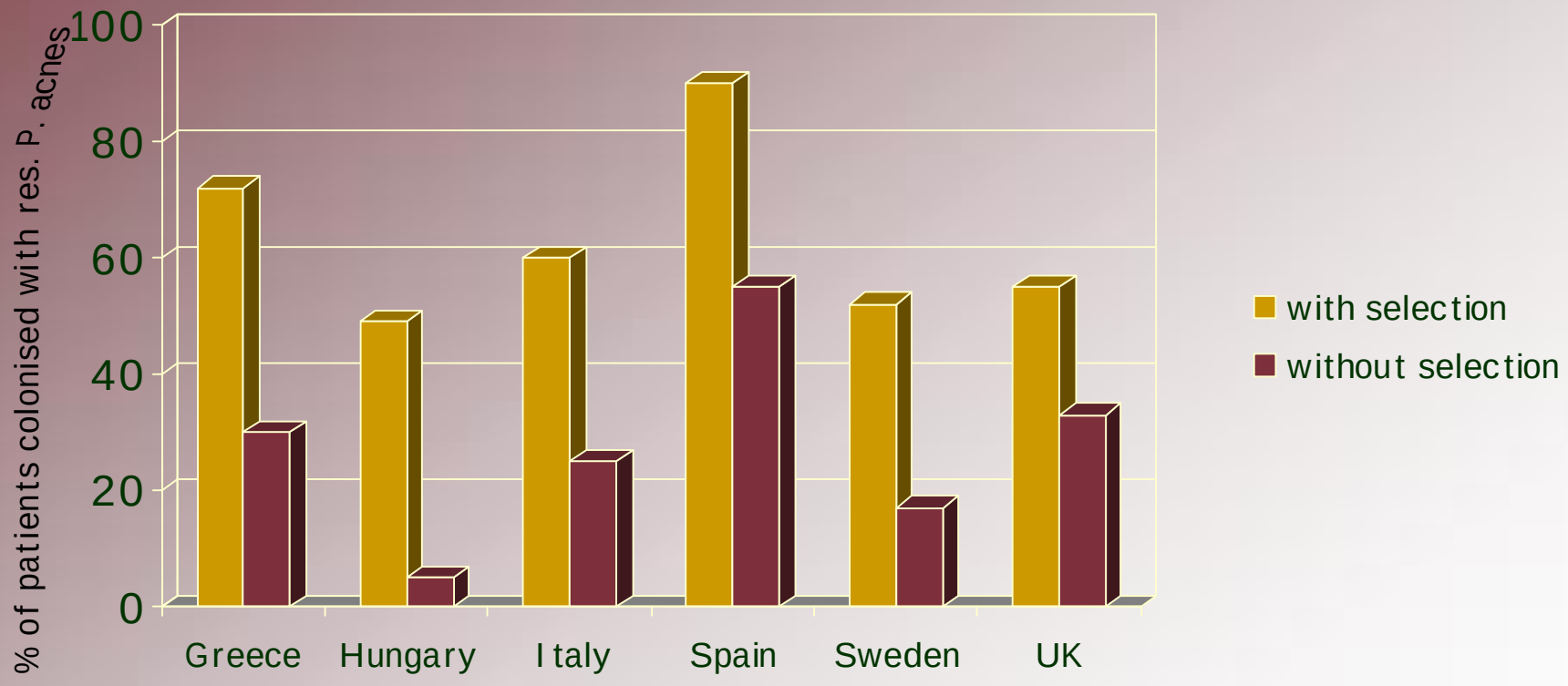
Eady E.A.: Dermatology 1998

Correlation between antibiotic usage and colonisation with resistant P.acnes

No. of patients treated with MLS antb. (n)	No. of patients (%) colonised with Eryt.-res. propionibacteria
Topical erythromycin (124)	93 (75%)
Topical clindamycin (71)	52 (73.2%)
Any oral macrolide (10)	6 (60%)
Any MLS antibiotic (202)	149 (73.4%)
No treatment for acne (196)	119 (60.7%)
All patients (622)	387 (62.2%)

Ross J.I. et al.: British Journal of Dermatology 2003

Problems to find antibiotic-resistant bacteria in the normal flora (erythromycin)



Propionibacterium acnes

Selection of quinolone resistance in anaerobes (Bacteroides)

- Quinolones with anti-anaerobic activity:
  - Temafloxacin (1990-1993)
  - Trovafloxacin (1997-)
  - Moxifloxacin (1998-)
- Development of trovafloxacin resistance according to different studies:
  - 1994-96 (Sydman et al.) 3-8%
  - 1997 (Hecht) 13%
  - 1998(Hecht) 15%
  - 2001 (Golan et al.) 25% (MIC: ↑ for moxifloxacin)
- Mutations at hotspot positions (Ser82Leu) of the *gyr*A gene (+ efflux pumps mediated resistance) has been confirmed by different authors

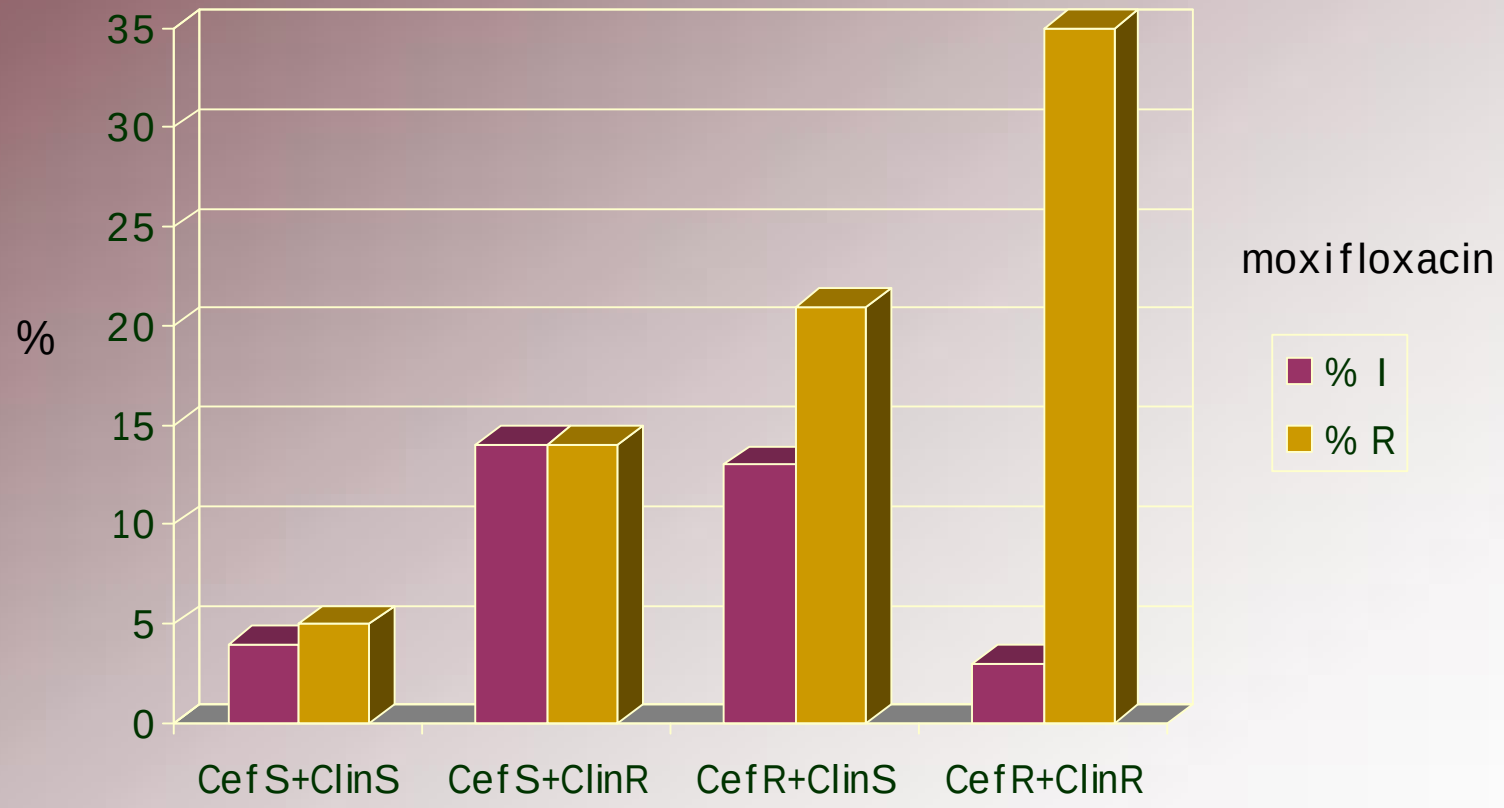
Oral administration of clinafloxacin promotes colonisation by quinolone-resistant Bacteroides *in faecal flora*

(Oh H., et al. Infection 2000)

Clinafloxacin was administered for 7 days P.O. 2x100 mg

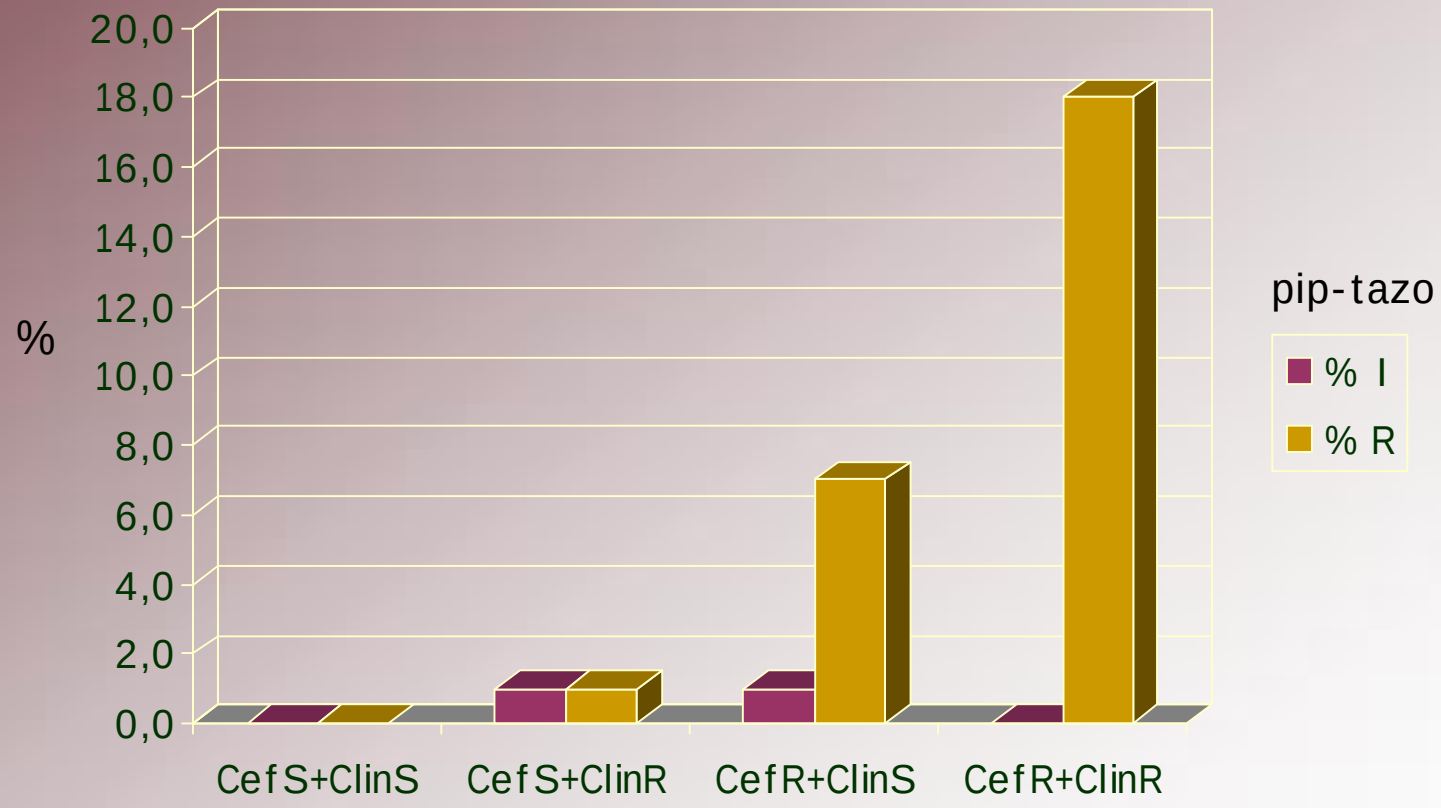
Bacteroides spp	MIC50	MIC90 (µg/ml)	Range
Day 0	0.5	8	0.064-32
Day 7	8	32	0.064-32
Day 21	1	16	0.064-32

Accumulation of resistance markers in anaerobes (Bacteroides)



Hedberg et al: based on the ESGARAB study

Accumulation of resistance markers in anaerobes (Bacteroides)



Hedberg et al: based on the ESGARAB study

Accumulation of resistance markers in anaerobes (Bacteroides)

- **All *Bacteroides* strains (16/1284) with MIC≥8 µg/ml for imipenem were resistant to at least one further antibiotic**
- **Despite the fact that *B. fragilis* seems to be more susceptible to antibiotics than other species**
  - **1 *B. fragilis* (UK) had high MICs for IPM, TZP, FOX and MTR**
  - **1 *B. fragilis* (CHE) had high MICs for IPM, TZP, FOX and CLI**
  - **1 *B. fragilis* and 1 *B. thetaiotaomicron* (SWE) had high MICs for IMP, TZP, FOX and CLI**
  - **1 *B. fragilis* (FRA) had high MICs for IPM, TZP, FOX and CLI, and was resistant to MFX as well**

ESGARAB study about *B. fragilis* group isolates in Europe (CMI 2002)

Simultaneous resistance to metronidazole, co-amoxiclav and imipenem in clinical isolate of *B. fragilis* (Turner et al. Lancet 1995)

- A 38-year old women underwent laparotomy to remove adhesions from the pelvic region. The history included recurrent urinary tract infections, treated several time with antibiotics.

- Fever developed 4 days postoperatively ⟶ amox/clav

- Peritonitis and small bowel obstruction on day 13 necessitated a new laparotomy. Many foetid abscesses were found below the diaphragm and in the pelvic region. These were removed and the abdominal cavity was drained.

Continuation, I

- The antibiotic chosen at that time was cephotaxime + metronidazole. Culture resalt of metrial taken intraoperatively revealed:
  - Coliform bacteria in high number
  - No B. fragilis or other anaerobic bacteria
  - Direct GLC positivity (anaerobic infection confermed)

- The patient temporarly improved, but the fever remained. The subphrenic abscess and empyema confirmed by X-ray led to an antibiotic change (imipenem+gentamycin+metronidazole).
- was isolated from the blood culture this time.

Continuation, II

- The empyema persisted on the right side after 2 week. *B. fragilis* was again isolated from a sample taken during renewed drainage (strain no 2.)
- The resistance data justified a further therapy changes clindamycin+ gentamycin
- Despite the developed vesico-vaginal fistula (dealt with later), the patient was discharged 9 weeks postoperatively

Was the *B. fragilis* strains isolated from the blood and the pus the same, but with altered resistance?

- Yes, because
  - their biotypes were identical (VITEK, BioMerieux);
  - both strains carried the nim gane and the iS eement necessary fro the expression of the gene „upstream” from the gene;
  - molecular typing proved the identity of the two strains.

What was the cause of the paersistant anaerobic infection?

B. fragilis	MIC (µg/ml)			
	Imi (≤4)*	Amox (≤2)	Amox/clav (≤2/1)	Metr (≤8)
Strain 1 (blood)	4	>128	16	8
Strain 2 (pus)	32	>128	64	0.25

*sensitivity breakpoint (CLSI)

What can we do to prevent the spread of antibiotic resistance among the bacterial population?

- As a doctor?
- As a patient?
- As an opinion leader in health care ?