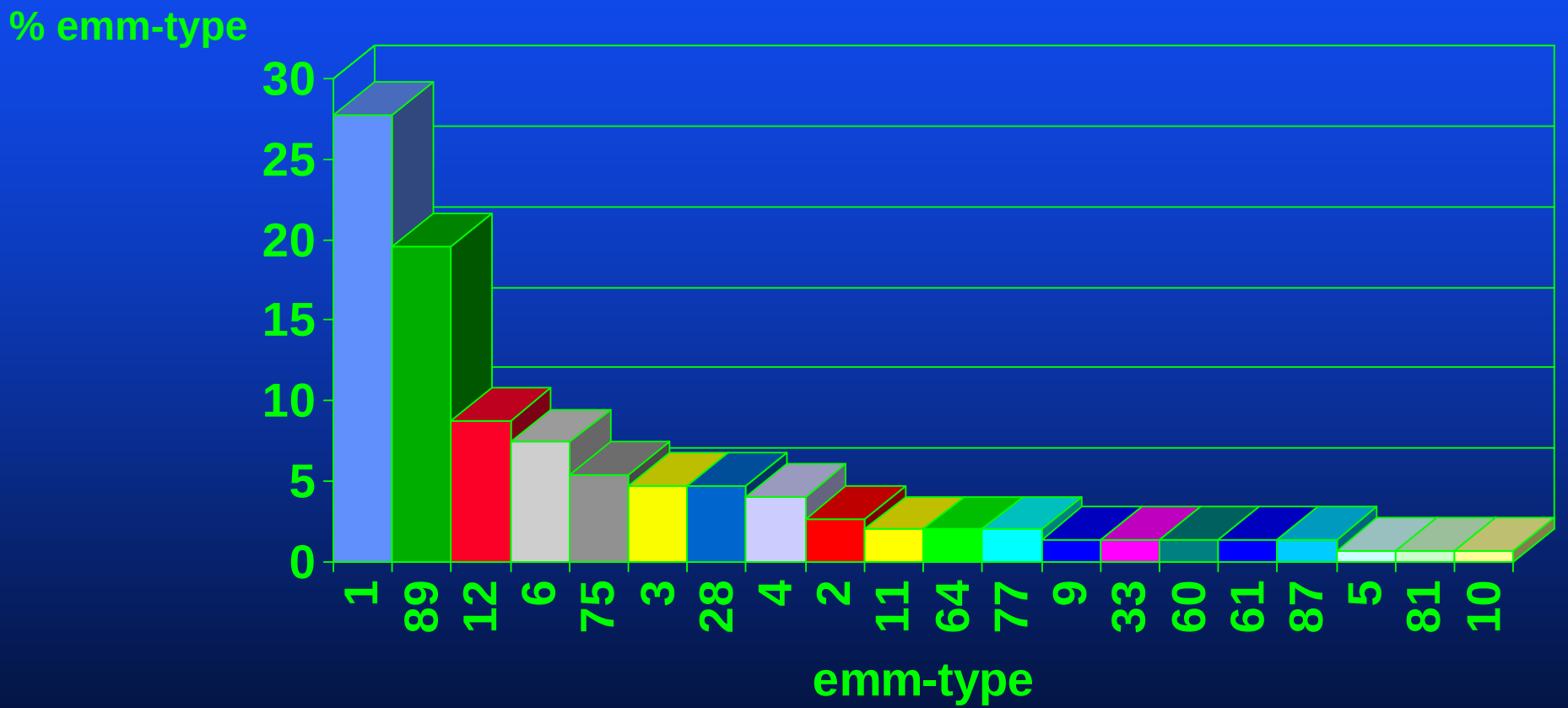
GAS Isolates Collected at the Belgian Reference Centre for GAS

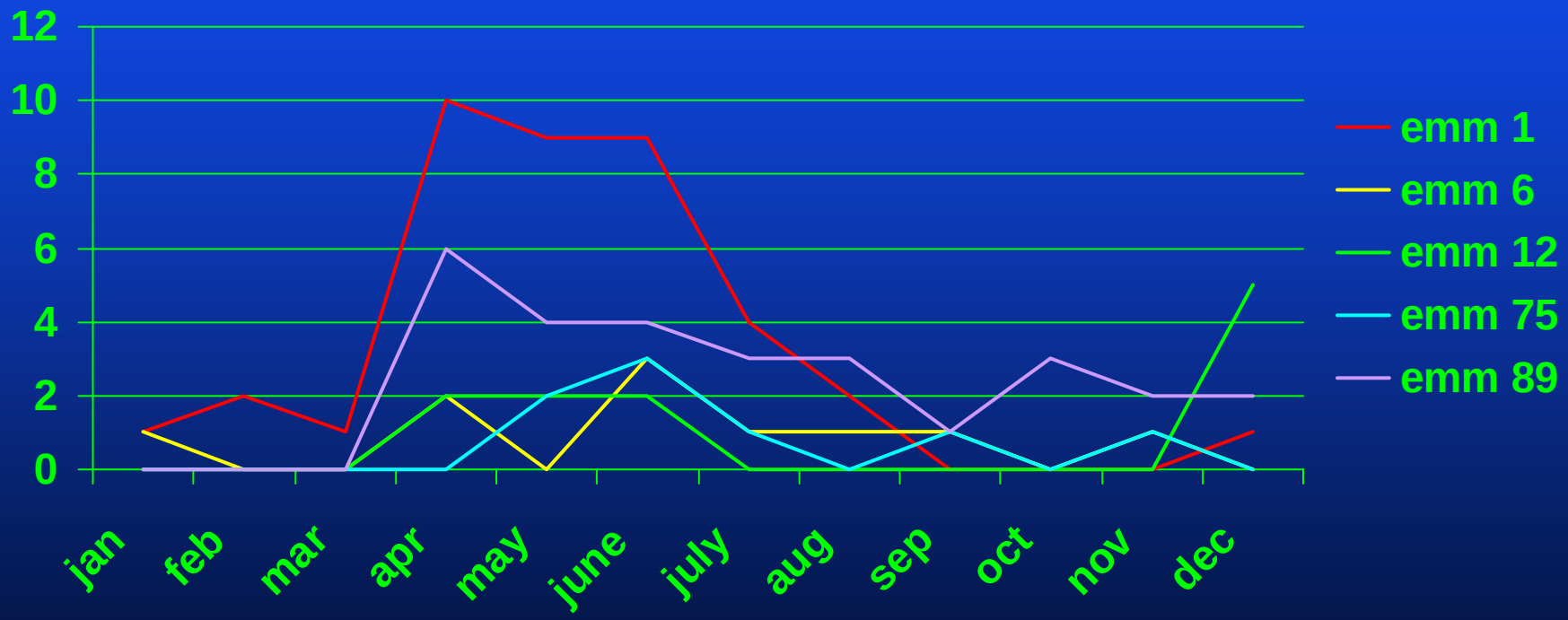
No of GAS	Year												
	1993	1994	1995	1996	1996	1997	1998	1999	2000	2001	2002	2003	2004
Sterile sites	80	138	32	49	49	33	18	40	76	66	75	93	148
Non-sterile sites	299	742	34	80	80	72	25	507	445	750	1312	1348	1326

Age distribution GAS Isolated in 2004

Prevalence of emm-types of GAS Isolated from Sterile Sites in 2004



Prevalence of emm-types (> 5%) of GAS Isolated from Sterile Sites in 2004



Clonality of GAS Isolated from Sterile Sites in 2004 According to emm-type

PFGE type No.	Emm-type					
	emm 1	emm 6	emm 12	emm 75	emm 89	Total
3	41					41
4				1		1
5		11				11
6			2			2
15				3		3
49				1	29	30
57			9			9
1035			2			2
1042				1		1
Total	41	11	13	6	29	100

Susceptibility to MLS of GAS by emm-type Isolated from Sterile Sites in 2004

Emm-type (No.)	Antibiotic							
	Erythromycin				Clindamycin			
	Range	Mic50	Mic90	% R	Range	Mic50	Mic90	% R
1 (40)	0.047-0.125	0.094	0.094	0	0.047-0.50	0.094	0.125	0
6 (11)	0.032-0.094	0.064	0.064	0	0.064-0.125	0.064	0.125	0
12 (9)	0.047-16	0.094	12	22.2	0.064-0.19	0.125	0.19	0
75 (8)	0.064-8	0.094	0.125	12.5	0.064-0.19	0.064	0.19	0
89 (28)	0.047-0.19	0.064	0.125	0	0.032-0.25	0.094	0.125	0

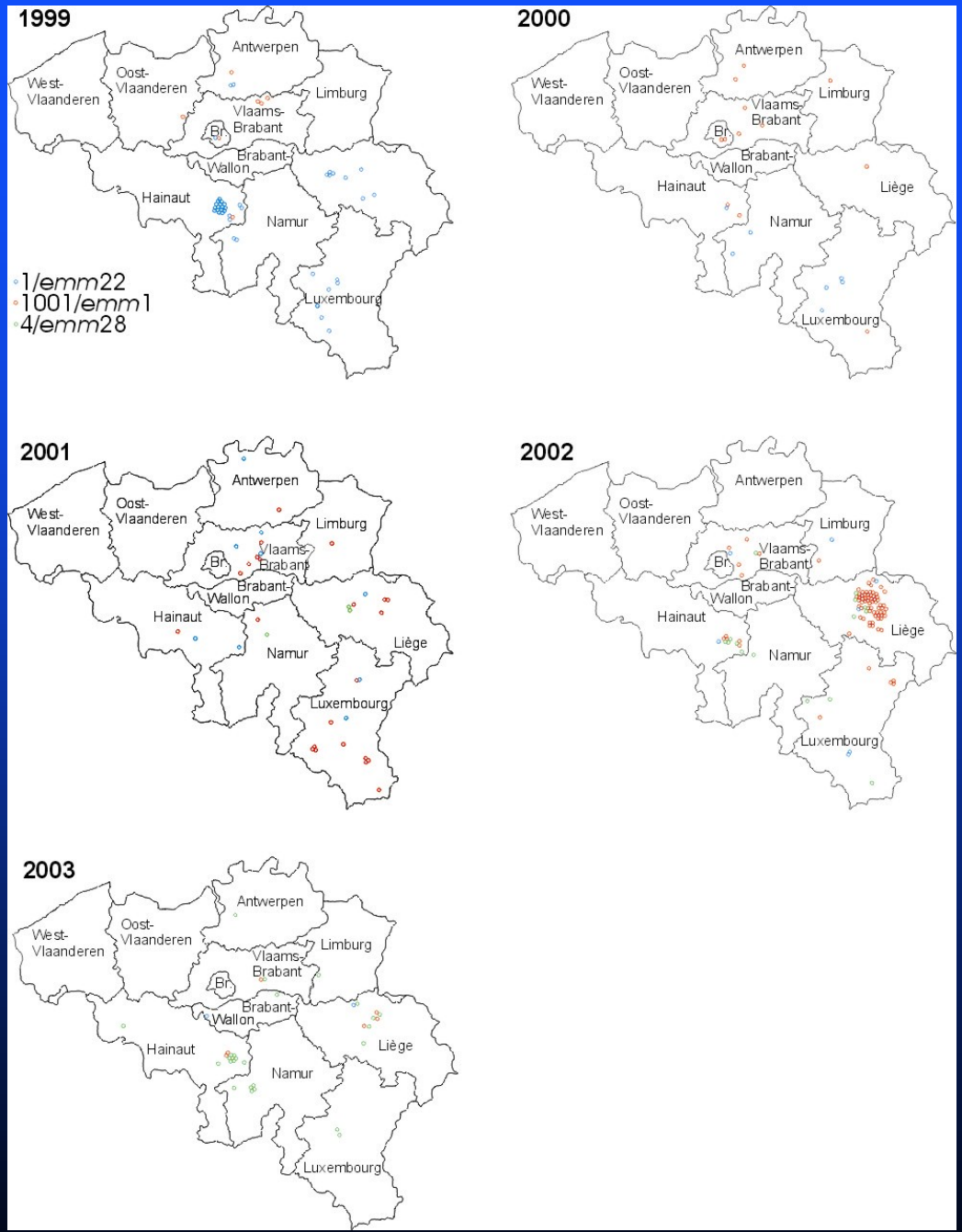
Yearly Prevalence of GAS Throat Isolates Screened and of Macrolide-resistant GAS Distributed by Age Group in Belgium

	1999	2000	2001	2002	2003
GAS isolates screened	**598**	**336**	**633**	**1226**	**1073**
no. of adults with GAS (mean age: 34.7; range: 17 years - 91 years)	220/598 (36.7%)	144/336 (43.1%)	245/633 (38.7%)	469/1226 (38.2%)	453/1073 (42%)
No. of children with GAS (mean age: 7.2; range: 3 months - 16.9 years)	357/598 (59.6%)	172/336 (51.2%)	367/633 (58.0%)	675/1226 (55.0%)	552/1073 (51%)
Macrolide-resistant GAS	**81/598 (14%)**	**41/336 (12%)**	**73/633 (12%)**	**215/1226 (18%)**	**96/1073 (9%)**
No. of adults with macrolide-resistant GAS	23/598 (4%)	16/336 (5%)	29/633 (5%)	82/126 (7%)	38/1073 (4%)
No. of children with macrolide-resistant GAS	56/598 (9%)	22/336 (7%)	44/633 (7%)	126/1226 (10%)	50/1073 (5%)
No. of macrolide-resistant GAS of the cMLS phenotype (prevalence, proportion)	49/81 (8%;60%)	10/41 (3%;24%)	28/73 (4%;38%)	68/215 (6%;31%)	54/96 (5%;56%)
No. of macrolide-resistant GAS of the M phenotype (prevalence, proportion)	32/81 (5%;40%)	29/41 (9%;71%)	39/73 (6%;53%)	141/215 (12%;65%)	38/96 (4%;40%)
No. of macrolide-resistant GAS of the MLS phenotype (prevalence, proportion)	-	2/41 (1%;5%)	6/73 (1%;8%)	7/215 (1%;3%)	4/96 (0.4%;4%)

Proportion of Macrolide-resistant GAS among the Total Throat GAS Isolated from 10 Belgian Provinces, 1999-2003

Province	No. of macrolide resistant strains				
	1999	2000	2001	2002	2003
Antwerpen	5/111 (5%)	4/96 (4%)	3/121 (2%)	3/170 (2%)	5/154 (3%)
Brabant-Wallon	0/5	0/25	0/6	3/29 (10%)	8/33 (24%)
Brussels Capital Dist	2/21 (10%)	2/8 (25%)	1/4 (25%)	2/49 (4%)	3/79 (4%)
Hainaut	29/84 (35%)	9/51 (18%)	8/60 (13%)	17/124 (14%)	26/228 (11%)
Liège	12/96 (13%)	1/13 (8%)	12/99 (12%)	132/333 (40%)	26/138 (19%)
Limburg	1/25 (4%)	1/13 (8%)	1/42 (2%)	7/95 (7%)	5/56 (9%)
Luxembourg	13/58 (22%)	13/60 (22%)	31/145 (21%)	25/160 (16%)	8/76 (11%)
Namur	2/36 (6%)	3/12 (25%)	6/25 (24%)	3/56 (5%)	8/82 (10%)
Oost-Vlaanderen	1/11 (9%)	1/6 (17%)	0/8	0/11	0/12

Temporal and Geographical Distribution of the Three Major Macrolide-resistant GAS Clones in Belgium



Temporal Changes in Prevalence of FQ Non-susceptible *S. pyogenes* Isolated from Tonsillopharyngitis Patients in Belgium

Year	1999	2000	2001	2002
Fluoroquinolone non-susceptible *S. pyogenes*	28/598 (4.6%)	29/633 (8.6%)	59/633 (9.3%)	36/1226 (2.9%)
No. of adults with fluoroquinolone non-susceptible *S. pyogenes*	17/220 (7.8%)	13/145 (9.0%)	19/245 (7.8%)	11/469 (2.3%)
No. of children with fluoroquinolone non-susceptible *S. pyogenes*	11/357 (3.1%)	14/172 (8.1%)	38/368 (10.4%)	21/675 (3.1%)

Temporal Changes in *emm* Type Distribution of Fluoroquinolone Non-susceptible and Susceptible *S. pyogenes (I)*

Year		1999	2000	2001	2002
emm-type distribution of fluoroquinolone non-susceptible *S. pyogenes*	*emm*6	26/28 (92.8%)	28/29 (96.5%)	47/59 (79.6%)	14/36 (38.8%)
	*emm*75	0/28 (0.0%)	0/29 (0.0%)	7/59 (11.8%)	16/36 (44.4%)
	others	2/28 (7.1%)	1/29 (3.4%)	5/59 (8.4%)	6/36 (16.6%)

Temporal Changes in *emm* Type Distribution of Fluoroquinolone Non-susceptible and Susceptible *S. pyogenes (II)*

Year		1999	2000	2001	2002
distribution of fluoroquinolone susceptible *S. pyogenes*	*emm*1	13/172 (7.6%)	16/84 (19.0%)	37/171 (21.6%)	70/200 (35.0%)
	*emm*4	22/172 (12.8%)	15/84 (17.9%)	27/171 (15.8%)	21/200 -type (10.5%)
	*emm*22	37/172 (21.5%)	13/84 (15.5%)	19/171 (11.1%)	8/200 (4.0%)
	*emm*6	1/172 (0.5%)	0/84 (0.0%)	1/171 0.6%)	1/200 0.5%
	*emm*75	2/172 (1.2%)	3/84 (3.6%)	2/171 1.2%)	0/200 (0.0%)

Amno Acid Substitutions in ParC and ParE Associated with each emm serotype and PFGE type among the clonal and unique fluoroquinolone non-susceptible Isolates Analysed. All Clonal Isolates Analysed from each PFGE Cluster Carried the Same set of Substitutions in ParC and ParE (I)

M type	PFGE cluster	Frequency (n=152)	Predicted ParC substitutons: no. of isolates anaysed	Predicted ParE substitutions*
emm6	5	113 (74.3%)	S79A;n=25	A378T
	61	1 (0.7%)	S79A; n=1	A378T
	9	1 (0.7 %)	S79A;n=1	A378T
emm75	15	21 (13.8%)	S79F, D91N; n=13	-
	39	2 (1.3%)	S79Y, D91N; n=2	-
emm28	2	3 (2.0%)	S79Y,D91N, n=3	-
	4	1 (0.7%)	S79F, D91N; n=1	-
	73	1 (0.7%)	D91N; n=1	ND

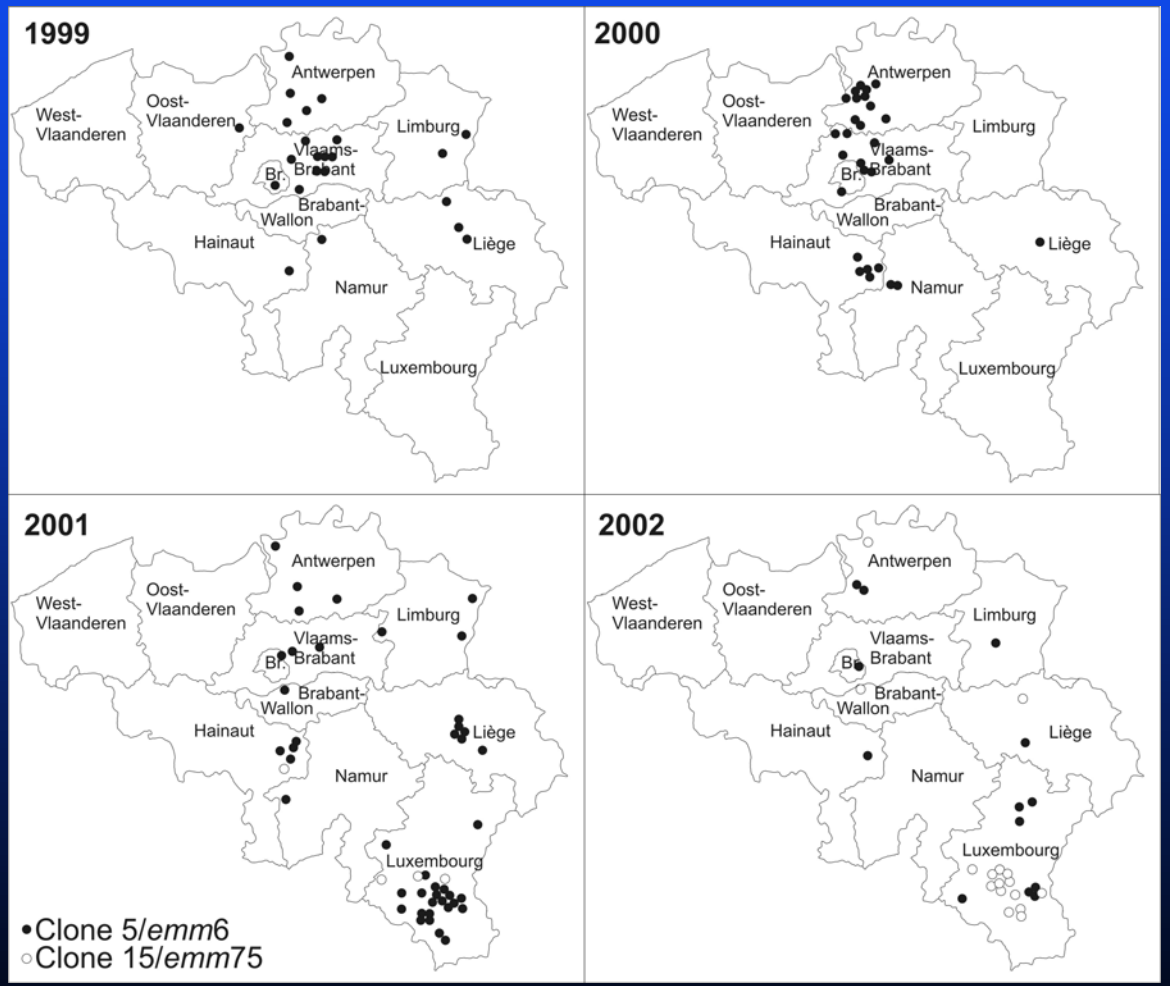
*The number of isolates sequenced in ParE are the same as in ParC except for cluster 73.

Amno Acid Substitutions in ParC and ParE Associated with each emm serotype and PFGE type among the clonal and unique fluoroquinolone non-susceptible Isolates Analysed. All Clonal Isolates Analysed from each PFGE Cluster Carried the Same set of Substitutions in *ParC* and *ParE* (II)

M type	PFGE cluster	Frequency (n=152)	Predicted *ParC* substitutons: no. of isolates anaysed	Predicted ParE substitutions*
emm 12	57	2 (1.3%)	S79F, S140P, D91N; n=2	-
	6	1 (0.7%)	S79F, A121V; n=1	-
emm 1	101	1 (0.7%)	S79F; n=1	-
emm 4	102	1 (0.7%)	S79A; n=1	A378T
emm 9	49	1 (0.7%)	D91N, S140P; n=1	Q360D
*emm*22	1	1 (0.7%)	D91N, G128D, S140P; n=1	-
emm 76	32	1 (0.7%)	S79Y, A121V; n=1	-
Non-typable	72	1 (0.7 %)	ND	ND

*The number of isolates sequenced in ParE are the same as in ParC except for cluster 73.

Temporal and Geographical Distribution of the Two Major Fluoroquinolone Non-susceptible *S. pyogenes* Clones in Belgium. Closed circles: clone 5/*emm*6, Open Circles: Clone 15/*emm*75



CORRELATION BETWEEN MACROLIDE RESISTANCE AND INVASIVENESS

Facinelli et al, Lancet 2001.

GAS isolates from children with pharyngitis (Italy, 1997-1998);

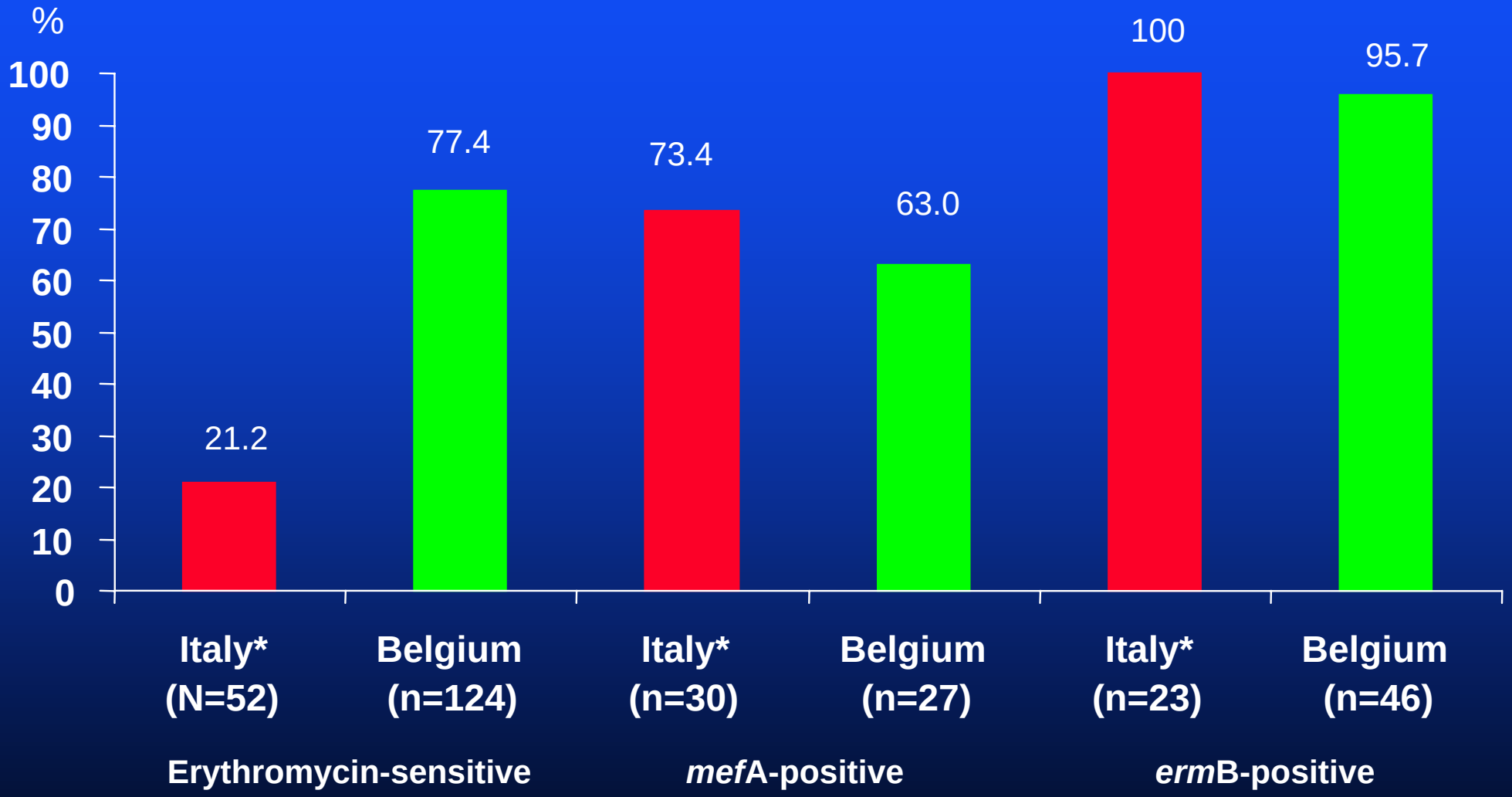
74 ery-R / 52 ery-S:

- **Presence of prtF1 gene**
- **Cell invasion (A549 cellen)**

Resistance phenotype	n	Resistance genotype					*prtF1* gene		Cell-invasion efficiency		
		ermB +*mefA*	*ermB*	*ermTR* +*mefA*	*ermTR*	*mefA*	Positive	Negative	High	Low	Non-invasive
Erythromycin-resistant											
All resistant	74	14 (19%)	9 (12%)	4 (5%)	17 (23%)	30 (41%)	66 (89%)	..	59 (80%)	7 (10%)	0
							..	8 (11%)	0	3 (4%)	5 (7%)
cMLS	10	7	..	..	..	..	7	0	7	0	0
			3	..	..	..	3	0	3	0	0
iMLS-A	13	7	..	..	..	..	7	0	7	0	0
		..	6	..	..	..	6	0	5	1	0
iMLS-B	13	..	..	1	..	..	1	0	1	0	0
		..	..	..	12	..	12	0	11	1	0
imLS-C	8	..	..	3	..	..	3	0	3	0	0
		..	..	..	5	..	5	0	5	0	0
M	30	..	..	..	..	30	22	..	17	5	0
		..	..	..	..	..	..	8	0	3	5
Erythromycin-susceptible	52	..	..	..	..	..	11 (21%)		5 (10%)	6 (12%)	0
								41 (79%)	0	15 (29%)	26 (50%)

Characteristics of erythromycin-resistant and erythromycin-susceptible strains of group A streptococci

Detection of *prt*F1 Gene in Group A Streptococci

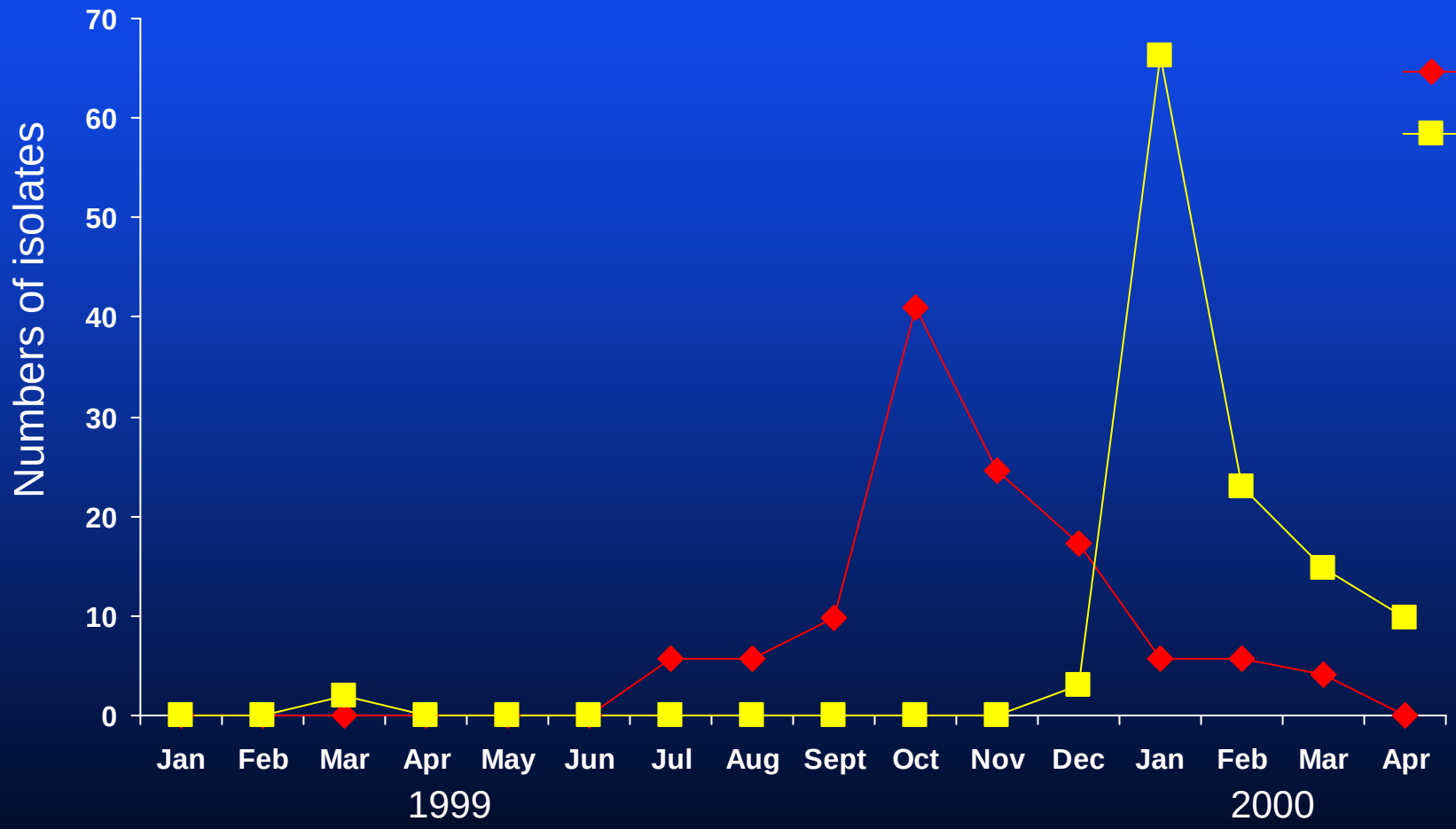


41st ICAAC*, Chicago (IL), 2001, Abstract #322

Association between Erythromycin-Resistance and Number of *RD2* Repeats of the Internalization-Associated Gene *prt*F1 in GAS

Erythromycin genotype	Number (%) of RD2 repeats						Total
	0	1	2	3	4	5	
Sensitive	28 (22.6)	16 (12.9)	25 (20.2)	19 (15.3)	35 (28.2)	0	123
Resistant							
*mef*A	10 (37.0)	6 (22.2)	2 (7.4)	2 (7.4)	5 (18.5)	2 (7.4)	27
*erm*B	2 (4.3)	1 (2.2)	3 (6.5)	1 (2.2)	7 (15.2)	32 (69.6)	46
Total	39 (19.9)	23 (11.7)	30 (15.3)	22 (11.2)	47 (24.0)	34 (17.3)	196

Dynamic Epidemiology of Group A Streptococcal Serotypes Associated with Pharyngitis



Kaplan et al., The Lancet; 358: 1334-37, 2001

Invasive Index of Group A Streptococcal Genotypes

Shulman et al, CID 2004; 39:325-32.

emm type	Percentage of isolates of stared type Pharyngitis (n=1975)	Invasive (n=1061)	Invasive index value
1	18.9	8.2	0.96
12	18.4	8.4	0.46
28	10.6	7.9	0.75
4	8.4	2.4	0.29
3	7.8	10.2	1.31
2	6.6	2.4	0.36
6	5.8	1.5	0.26
89	4.6	5.5	1.20
77	3.8	3.6	0.95
22	2.8	2.4	0.86
44/61	2.1	1.0	0.48
5	1.4	1.7	1.21
75	3.8	2.9	0.76
82	0.4	5.9	14.75
11	1.0	3.4	3.40
114	0.1	2.6	26.00
73	0.3	2.2	7.33

Comparison of Group A Streptococcal Isolates from Individuals in an Aboriginal Island Community in Australia with Isolates of the Same *emm* Type from Non-Australian Sources

No. of shared alleles	No. of *emm* types found	emm types represented
5-7	10	9, 14, 42, 44/61, 49, 58, 65/69, 85, 89 and stD633
3-4	6	44/61, 52, 80, 69, 90, and 101
0-2	13	4, 14, 22, 25, 52, 60, 65/69, 70, 81, 97, 110 and 114

McGregor et al, JID 2004;189: 717-23

Group A Streptococcal Virulence Antigens

Antigen	Characteristic/function	Variable structure/repeats	CWP/secreted
SclA	Collagen-like, adherence?	Yes	CWP
SciB	Collagen-like, adherence?	Yes	CWP
GRAB	2-macroglobulin binding	Yes	CWP
MtsA	Metal ion transport	No	CWP
EndoS	IgG endoglycosidase	Unlikely	Secreted
IdeS	IgD degrading enzyme	Unlikely	Secreted
SpeB	Cysteine proteinase	No	Secreted

Note CWP, cell wall protein

Akesson et al, JID 2004;189: 797-804

Mean ELISA Indexes against Group A Streptococcal (GAS) Antigens in Blood Donors and in Patients with Invasive GAS Infection

Antigen	Subject group					
	Blood donors (n=80)	Patients with invasive GAS infection (n=70)	*P*	Patients with invasive GAS infection but otherwise healthy (n=43)	Patients with invasive GAS infection with underlying chronic disease (n=15)	*P*
SclA	0.22	0.49	<.05	0.39	0.69	<.05
SclB	0.46	0.83	<.05	0.70	1.05	<.05
GRAB	0.44	0.92	<.05	0.72	1.31	<.05
MtsA	0.51	0.88	<.05	0.64	1.22	<.05
EndoS	0.98	1.11	.23	0.96	1.50	<.05
IdeS	0.68	1.24	<.05	1.01	1.65	<.05
SpeB	0.53	1.02	<.05	0.82	1.41	<.05

Akesson et al JID 2004; 189: 797-804

Role of Host Genetic factors: Haplotypes Associated with Different Manifestations of Invasive GAS Infections

Manifestation of invasive GAS infection	Haplotypes with protective effect	Haplotypes with predispositional effect
Severe systemic disease	DRB1*1501/DQB1*0602	DRB1*14/DQB1*0503
NF	DRB1*03/DQB1*0201	DRB1*11/DQB1*0301*
Severe systemic disease in the presence of NF	DRB1*1501/DQB1*0602	DRB1*07/DQB1*0201*

* The DRB1*11/DQB1*301 haplotypes showed only a trend towards an association with riks for NF. The trend for an association of the DRB1*07DQB1*0201 haplotype with risk for SSD in the presence of NF was functionally validated.

Proportion of emm-types (> 5%) According to Site of Isolation in 2004

Site of isolation	Emm-type (>5 %)					Total
	1	89	12	6	75	
Blood isolates	27 (86%)	17 (59%)	9 (69%)	6 (55%)	4 (50%)	63
Other sterile Sites	14	12	4	5	4	39
Total	41	29	13	11	8	102