Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

ELSEVIER

International Journal of Antimicrobial Agents 18 (2001) 9–17

INTERNATIONAL JOURNAL OF
Antimicrobial
Agents

www.ischemo.org

Original article

Bacteriological and clinical efficacy of various antibiotics used in the treatment of streptococcal pharyngitis in Italy. An epidemiological study

G. Rondini [a], C.E. Cocuzza [b], M. Cianflone [b], A. Lanzafame [b], L. Santini [b], R. Mattina [b,*]

[a] *Div. Pat. Neonatale OSM IRCCS, University of Pavia, Pavia, Italy*
[b] *Institute of Medical Microbiology, University of Milan, Via Pascal, 36, 20133 Milan, Italy*

Received 26 January 2001; accepted 6 April 2001

Abstract

A total of 123 community paediatricians and 23 microbiology laboratories studied the clinical and bacteriological efficacy of treatment of group A streptococcal pharyngitis in Italy. Of 1065 patients, from whom *Streptococcus pyogenes* was isolated, 723 returned to follow up and of these 138 (19%) still had a positive throat culture. The erythromycin resistance (ER) rate was 23.7% with resistance phenotype distribution of: 31.7% constitutive (CR), 26.6% inducible (IR) and 41.7% efflux pump (M) resistance phenotype. All strains were susceptible to the β-lactam agents tested. CR strains were highly resistant to all 14, 15 and 16 membered macrolides with the exception of rokitamycin which showed activity against 37.8% of isolates. All phenotype M and some IR isolates were susceptible to clindamycin, rokitamycin, josamycin and spiramycin; clarithromycin was active against a small percentage of strains belonging to the IR and M phenotype. Bacterial eradication was found in 85.5, 78.7 and 75.8% of the penicillin, macrolide and cephalosporin treated groups. Genotyping of strains showed that 8.7% of the 19% of cases classified as 'failed bacterial eradication' were due to recolonization with a different isolate, observed exclusively among β-lactams treated patients. Clinical cure was achieved in a high percentage of cases, irrespective of the antibiotic prescribed, with the best clinical efficacy being found following therapy with amoxycillin and clarithromycin (90.9%).

Keywords: Clarithromycin; β-lactam; Streptococcal pharyngitis

1. Introduction

Pharyngotonsillitis is reported to be caused by many viruses and bacteria; among them, group A β-haemolytic streptococcus (*Streptococcus pyogenes*) is the most frequent bacterial cause. About 30% of children with sore-throat have a positive culture for this organism and antibiotic treatment is recommended [1,2]. Rhinovrius, adenovirus and coronavirus are responsible for the other 70% [3].

Acute pharyngotonsillitis caused by group A β-haemolytic streptococci is often clinically indistinguishable from a viral infection; therefore an accurate microbiological investigation is important. In untreated group A infection the organism can persist and cause serious suppurative and non-suppurative complications

The aim of antibiotic therapy should therefore be not only clinical cure but also bacteriological eradication.

Penicillins are still generally considered the drugs of choice [4], but they have been found to fail in eradicating group A streptococci in 5–30% of children with streptococcal pharingotonsilitis, even though the isolated strains were shown to be susceptible. Recently it has been demonstrated [5] that some group A strepto-

* Corresponding author. Tel.: +39-02-6601233; fax: +39-02-26601218.
E-mail address: roberto.mattina@unimi.it (R. Mattina).

PII: S0924-8579(01)00342-9

coccal strains can enter respiratory epithelial cells, where they became inaccessible to those antibiotics unable to penetrate the cell membrane, such as penicillins. These findings and the increasing incidence of *Mycoplasma pneumoniae* and *Chlamidia pneumoniae* as pathogens have favoured the spread of macrolide use [1,6]. Erythromycin has been the principal alternative for penicillin-allergic patients [7,8].

Less than 5% of *Streptococcus pyogenes* isolates are generally reported as resistant to macrolide antibiotics [9,10]. A marked increase in the incidence of resistant strains has been reported in a limited number of countries, namely Australia [11], Finland [12], Sweden [13], the UK [14], Japan [15,16] and Taiwan [17].

From the late eighties an appreciable resistance to macrolides has also been described in Italy in patients with pharyngotonsillitis and scarlet fever [18–21]. A recent national survey confirmed a dramatic increase in macrolide resistance in most areas of the country [22]. In 1993, the incidence of macrolide resistance was 5.1%, rising to 25.9% in 1995 with some centres showing peaks of 30–40%. Other Italian studies in the 1990 s showed similar results [23,24].

There are two recognized mechanisms of resistance to macrolide antibiotics in streptococci: target modification and an efflux system. These mechanisms give rise to three possible phenotypes: constitutive, inducible and the so called newly resistant or M phenotype. In strains exhibiting the constitutive and inducible phenotype, resistance is due to the methylation of the bacterial ribosome; M phenotype isolates are found to have an efflux system for 14 and 15 membered macrolides, apparently distinct from the efflux system described for erythromycin-resistant staphylococci [25,26].

The aim of this study was to follow up children with streptococcal pharyngotonsillitis treated with antibiotics chosen freely by their paediatricians and to evaluate both clinical cure and microbiological efficacy of different therapies. Clinical cure and bacteriological eradication were determined and their correlation evaluated. In order to distinguish failed eradication from recolonization after treatment; all strains isolated before and at follow up were genotyped.

2. Materials and methods

2.1. Study design

The study was planned as an open multicentre clinical-microbiological study to evaluate susceptibility of Streptococcus β-haemolytic Group A (*S. pyogenes*), isolated from pharyngeal swabs in children with acute pharyngotonsillitis, aged from 3 to 14 years.

A total of 23 Italian microbiological centres (local microbiological centres) and 123 community paediatricians were involved. Patients who had received antibiotic therapy during the 2 weeks prior to the study were excluded.

We aimed to include at least 500 paediatric patients with streptococcal pharyngotonsillitis who had pharyngeal cultures before and after antibiotic treatment.

The investigation was co-ordinated by the Neonatal Pathology Department of Pavia University and by the Microbiology Institute of Milan University.

2.2. Patients

During the first visit, the patient's paediatrician would complete a case-report-form where history, signs and symptoms of the suspected infection and the prescribed antibiotic therapy would be registered. Clinical diagnosis of bacterial pharyngotonsillitis would be established taking into account the Breese score [27]. Antibiotic therapy would be chosen freely by the physician. A pharyngeal swab was taken before any therapy and, together with the case-report form, sent to the local microbiological centre for culture. The isolated strains were stored in glycerol at −80°C and sent to the Central Laboratory. If the microbiological culture was negative, the antibiotic therapy was stopped.

If culture was positive for group A streptococci, a second visit 7–10 days after the last day of antibiotic treatment was organized and the paediatrician would complete a second case-report-form with details of antibiotic therapy and clinical evaluation of the case. A second pharyngeal swab would be taken and sent to the local microbiological centre for identification.

Clinical judgement would be used to describe the patient as clinically cured, improved or exhibiting treatment failure. The patients were said to be clinically cured if all symptoms had disappeared at follow-up, improved if one symptom even if minor, except for fever, was still present and classified as treatment failure if fever or more than one symptom were still present.

2.3. Microbiological design

At the local microbiology centre, streptococcal strains were isolated, identified and disc susceptibility test to various antibiotics performed. The strains, labeled with coded identification numbers and stored in plates or frozen at −20°C (in Brain–Heart–Infusion broth added with glycerol 8%), were sent every 15 days to the central laboratory, with the case-report-forms. At the central laboratory all strains were re-identified, and the MIC determined to erythromycin, azithromycin, clarithromycin, roxithromycin, rokitamycin, josamycin, spiramycin, clindamycin, amoxycillin, amoxycillin/clavulanic acid, cefotaxime and tetracycline. The phenotypes of the erythromycin resistant strains were determined and genotypes of all isolates of cases positive at

follow-up, in order to distinguish between non-eradication and recolonization.

2.4. Isolation and identification

Pharyngeal swabs were immediately cultured on Columbia CNA-based blood agar plates containing 5% sheep blood (BioMérieux, Marcy-l'Etoile, France). Strain identification was performed with bacitracin discs (Difco Laboratories, Detroit, MI) and by either a latex agglutination assay (Streptex, Wellcome, Dartford, UK) or a latex-agglutination grouping kit (StreptoSlide, Dioesse Diagnostica Senese Srl).

2.5. Disc susceptibility test

The susceptibility of *S. pyogenes* isolates was initially evaluated by a disc diffusion method, according to the NCCLS Performance Standard 1995 [28]. All antibiotic discs were supplied by Oxoid (Unipath, Basingstoke) except for azithromycin and rokitamycin (Becton Dickinson, Meylan, France).

3. Central laboratory

3.1. Reidentification

The strains were reidentified at the central microbiology laboratory by the method already described.

3.2. Susceptibility test-MICs determination

Minimum inhibitory concentrations of antibiotics were determined by the microdilution method described in the NCCLS guidelines 1995 [29]. Cation adjusted Mueller Hinton broth (Difco) was used, supplemented with 2% lysed horse blood (Oxoid) and containing doubling dilutions of the antibiotic to be tested.

The streptococcal isolates were thawed, inoculated in BHI broth and incubated at 37°C for 6–8 h to allow bacteria to reach an exponential growth phase. Cultures were then diluted to obtain a final bacterial inoculum of 10^5 CFU/ml in a total volume of 100 μl/well. *S. pyogenes* ATCC 10383 was used as control.

The MIC values were recorded following 24 h incubation at 37°C. The MIC was defined as the antibiotic concentration in the first well, i.e. the well with the lowest antibiotic concentration, that did not support visible growth. Test results were interpreted according to NCCLS criteria [28] for those antibiotics for which breakpoints were available.

3.3. Resistance phenotypes

The macrolide-resistance phenotypes were determined for the erythromycin-resistant (ER) isolates by a modification of the method described by Seppala et al. [25] using two discs containing erythromycin (15 μg) and clindamycin (2 μg) discs (Oxoid). The discs were placed 7–10 mm apart on a blood agar plate (Mueller Hinton agar supplemented with 5% horse blood) inoculated with a bacterial suspension.

Following overnight incubation at 37°C, the inhibition zone around the two discs was observed. Blunting of the clindamycin zone of inhibition proximal to the erythromycin disc indicated an inducible resistance (IR) phenotype. Resistance to both erythromycin and clindamycin, with no blunting of the clindamycin inhibition zone, indicated a constitutive resistance (CR) phenotype. The resistance phenotype described as the M phenotype was characterized by resistance to erythromycin and susceptibility to clindamycin with no blunting of the zone around the clindamycin disc.

3.4. Genotype determination

3.4.1. Pulse-field gel electrophoresis (PFGE)

A bacterial culture (6 ml) of early stationary phase cells was pelleted by centrifugation at 4000 rpm for 15 min at 4°C, washed in 1 ml of PIV buffer (10 mM TRIS, pH 8, 1 M NaCl), and resuspended in 200 μl of the same buffer. Concentrations were adjusted to an A_{620} of 5 OD. This cell suspension was diluted 1:1 with 100 μl of 1.5% low-gelling-temperature agarose (Pharmacia Biotech, Uppsala, Sweden) in PIV buffer. Agarose discs of 20 μl were prepared and allowed to solidify for 5 min at −20°C. The cells were lysed by incubation of the discs at 37°C for 3 h in 1 ml of EC lysis buffer (6 mM TRIS, pH 8, 1 M NaCl, 0.1 M EDTA, pH 8, 0.2% sodium deoxycholate, 0.5% polyoxyethylene-20-cetyl-ether and 0.5% sarkosyl) with 50 mg/l of RNAse A and 1 mg/ml of lysozyme (Sigma). The discs were incubated at 50°C for 17 h in ES buffer (0.5 M EDTA, pH 9) containing 1 mg/ml proteinase K; they were then washed four times with 14 ml of TE buffer (10 mM TRIS, pH 7.5, 1 mM EDTA, pH 8), with gentle agitation for 1 h. The agarose discs were now considered to contain purified DNA and stored in TE at 4°C. Prior to electrophoresis, the agarose discs were allowed to equilibrate in 1 ml of restriction buffer (6 mM TRIS, pH 8, 20 mM KCl, 6 mM $MgCl_2$ and 6 mM 2-mercrispectiveaptoethanol) at room temperature for 30 min, followed by restriction for 18 h at 25°C in 45 μl of restriction buffer containing bovine serum albumin (BSA; 100 μG/ml) and 480 U/ml *Sma*I (Pharmacia Biotech). The reaction was stopped with 10 μl of ES buffer and 5 μl of loading buffer.

Electrophoresis gels were prepared using 1% agarose (Pharmacia Biotech) in 0.5 × TBE buffer (44.5 mM TRIS-borate 1.25 mM EDTA). Discs containing the digested DNA were loaded in the gel and sealed with 0.75% low-gelling-temperature agarose. Two molecular-

weight markers were used: lambda ladder (50–1000 kb) and low-range (0.1–200 kb) Pulsed Field Gel Markers (Sigma).

Pulse-field gel electrophoresis was performed using a Gene Navigator System apparatus (Pharmacia Biotech). The running conditions were 112 V for 40 h at 11.3°C. Pulse times were 20 s for 15 h, 35 s for 7 h, 50 s for 15 h and 90 s for the last 3 h. Gels were visualized and photographed following ethidium bromide staining under UV light.

Interpretation of DNA restriction patterns generated by pulse-field gel electrophoresis used the guidelines proposed by Tenover et al. [30]. The DNA restriction pattern of isolates differing by more than three restriction fragments were considered to belong to different clonal types.

4. Results

4.1. Microbiological evaluation

Pharyngeal swabs were positive for *S. pyogenes* in 1065 cases at first visit (baseline) and in 138 cases at second visit (follow-up).

Antibiotic therapy was continued in all cases of positive pharyngeal swabs, using the paediatrician prescription for daily dosage and duration.

Table 1 shows the number of strains isolated in the laboratories of the participating centres. Of the total 1065 baseline isolates, 813 (76.3%) were erythromycin-susceptible (ES) and 252 (23.7%) were erythromycin-resistant (ER). Of these, 80 (31.7%) were of CR phenotype, 67 (26.6%) of IR phenotype and 105 (41.7%) of M phenotype.

All strains, irrespective of their susceptibility to erythromycin, showed MICs to amoxycillin, amoxycillin plus clavulanic acid and cefotaxime ranging from $\leq$ 0.0015 to 0.015 mg/l.

MIC distribution for all other tested agents, expressed as cumulative percentages and subdivided according to the different susceptibility phenotypes, is reported in Table 2. ES-strains were susceptible to all antibiotics tested, except to tetracycline to which 1.7% of these strains were found to be resistant. The 252 ER-strains were all susceptible to penicillins and cefotaxime, with variable tetracycline resistance. The 80 strains of CR phenotype were highly resistant to most

Table 1
Number of *S. pyogenes* strains isolated at first visit showing susceptibility and resistance to erythromycin and the distribution of different phenotypes

Centers	Total organsims (*n*)	Susceptible (*n*)	Resistant (*n*)	Phenotypes		
				CR[a]	IR[b]	M[c]
Abbiategrasso	6	6	–	–	–	–
Alba–Bra	30	23	7	–	2	5
Alessandria	39	24	15	9	2	4
Bassano del G.	36	30	6	–	3	3
Bologna	75	61	14	1	4	9
Casorate	45	42	3	–	–	3
Catania	191	157	34	11	11	12
Catanzaro	16	8	8	4	–	4
Cava dei Tirreni	31	27	4	–	–	4
Chiavenna	24	17	7	–	3	4
Imola	30	26	4	–	2	2
Lecco	55	40	15	6	3	6
Magenta	42	28	14	7	4	3
Manerbio	17	14	3	2	–	1
Mantova	86	41	45	10	12	23
Melegnano	70	54	16	9	3	4
Milano F.B.F.	28	23	5	2	3	–
Milano P.A.T.	48	36	12	3	2	7
Napoli	19	8	11	6	2	3
Pinerolo	11	2	9	1	5	3
Salerno	23	18	5	2	2	1
Sondalo	69	69	–	–	–	–
Vercelli	74	59	15	7	4	4
Total	1065	813	252	80	67	105

[a] CR, constitutive-resistance phenotype.
[b] IR, inducible-resistance phenotype.
[c] M, new resistance phenotype.

Table 2
Susceptibility of erythromycin-susceptible or -resistant *S. pyogenes* (%) to other antimicrobial agents

	MICs in mg/l													
	≤0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	≥128
(a) *S.pyogenes* erythromycin-susceptible strains ($n = 813$)														
Ery	0.4	54.2	45.0	0.4										
Roxit			10	86	4									
Clari	18.8	72.3	7.4	0.1										
Azitro		0.2	4.2	88.1	7.1	0.4								
Spira				0.4	15.7	83.9								
Josa				2.3	75.6	22.1								
Rokita			0.9	41.3	56.1	1.7								
Clinda		3.7	77.6	18.4	0.3									
Tetra			0.2	28.8	63.6	5.5		0.2		0.3	0.2	0	0.2	
(b) *S. pyogenes* erythromycin-resistant strains phenotype CR ($n = 80$)														
Ery														100
Roxit														100
Clari														100
Azitro														100
Spira														100
Josa													1.3	98.7
Rokita			1.3		6.2	5.0	22.5	27.5	10	10	2.5			15
Clinda													1.3	98.7
Tetra			8.8	16.2	1.3					2.5	31.2	23.8	16.2	
(c) *S. pyogenes* erythromycin-resistant strains phenotype IR ($n = 67$)														
Ery							5.9	6.0	1.5	4.5	2.9		4.6	74.6
Roxit									1.5	8.9	1.5	3.0	3.0	82.1
Clari						7.5	4.4	1.5	4.5	2.9		3.1	3.0	73.1
Azitro									1.5	10.4		3.0	5.9	79.2
Spira					19.4	4.4			3.0	4.5	6.0	2.9	3.1	56.7
Josa				17.9	5.9		4.6	1.5	2.9	4.5	2.9	4.6	3.0	52.2
Rokita			70.1	17.9	10.5	1.5								
Clinda			74.6	13.4	7.5									4.5
Tetra			25.3	40.0	17.9	1.5	1.5				4.4	9.0		
(d) *S. pyogenes* erythromycin-resistant strains phenotype M ($n = 105$)														
Ery								2.9	15.2	76.2	5.7			
Roxit									8.6	16.1	65.7	9.6		
Clari						0.9	2.9	8.5	62.9	21.9	2.9			
Azitro									4.8	75.2	20			
Spira				3.8	84.7	11.5								
Josa			11.4	79.0	9.6									
Rokita			98.1	1.9										
Clinda			99	1										
Tetra			7.6	15.2	11.5						20	39	6.7	

antibiotics; the 67 strains of IR phenotype were susceptible to clindamycin (95.3%), josamycin (31.3%), spiramycin (25%) while the 105 of M phenotype demonstrated 100% susceptibility to the same antibiotics. Azithromycin and roxithromycin were non-active whilst clarithromycin was found to be active against a small percentage of strains belonging to the IR and M phenotypes.

For rokitamycin, although no breakpoints are available, susceptibility results showed MICs ≤ 1 mg/l for 37.8% of CR strains and MICs ≤ 0.5 mg/l for all IR and M phenotype.

A higher incidence of ER-strains (Table 1) was registered at Pinerolo (81.8%), followed by Napoli (57.9%), Mantova (52.3%) and Catanzaro (50%); two centres (Abbiategrasso and Sondalo) did not isolate any resistant strains. Macrolide resistance over the Italian territory can thus be described as having a patchy distribution.

Of the total 723 cases who presented at follow-up, 585 (80.9%) showed bacterial eradication and 138 (19.1%) were still positive.

Table 3 reports clinical and bacteriological results following treatment with the major groups of antibiotics used in the treatment of these infections (713 patients). Penicillins and clarithromycin gave the best bacterial eradication results (88.4 and 85.3%, respectively) followed by amoxycillin plus clavulanic acid (83.6%) and oral cephalosporins (76.7%). Of the

macrolides, eradication percentages ranged from 59.4% with rokitamycin to 85.3% with clarithromycin. The highest percentage of microbiological failures (40.6%) was registered with rokitamycin, with 8 of the 13 non-eradicated microorganisms (25%) having MICs $\leq$ 1 mg/l at baseline and after therapy.

Table 4 shows bacterial eradication following macrolide treatment (for those agents with at least 30 evaluable patients) according to the susceptibility of the baseline isolate to erythromycin. Bacterial eradication of ES and ER baseline isolates was 92 and 60% following clarithromycin treatment; 83 and 37.5% following azithromycin and 56.5 and 66.7% with rokitamycin.

The phenotypes of ER isolates which were not eradicated following macrolide treatment were identical to those of baseline strains: this was for clarithromycin 7 CR, 2 IR and 3 phenotype M, for azithromycin 4 CR, 4 IR, and 2 phenotype M, for rokitamycin 1 CR, 2 IR and 1 phenotype M.

Interestingly in the azithromycin treated group, four erythromycin susceptible strains at baseline (MICs = 0.12 mg/l) were not eradicated and became ER following treatment: three exhibiting phenotype M (MICs 4–8 mg/l) and one with IR phenotype (MIC > 128 mg/l).

Genotype-identification of all strains isolated at baseline and at follow-up allowed us to distinguish non-eradication from recolonization for each antibiotic treatment group; the results are reported in Table 5. On the basis of genotyping it was found that all strains isolated at follow up belonging to the macrolides cefaclor, ceftibuten and ceftriaxone treated groups were confirmed 'true' failure of eradication. On the other hand for amoxycillin, amoxycillin and clavulanic acid, cefixime and cefuroxime axetil some cases of 'failed eradication' were shown to be due to recolonization with a different strain.

4.2. Clinical evaluation

Considering the 1065 patients positive at baseline, recurrent episodes of pharyngotonsillitis had been reported in 330 cases, 145 in ear infections, four in rheumatic fever and 29 in other correlated symptoms. Presence of concomitant diseases had been reported in 98 cases. 125 patients had been treated with antibiotics in the last 30 days. 97.1% of patients were aged under 12 years while 2.9 aged over 12 years of age.

A follow-up visit was performed after a mean period of 10 days from the end of antibiotic treatment.

Symptoms of infection, such as fever (> 37° C) and sore throat were evaluated at baseline and at follow-up.

Table 3 gives the major antibiotic therapies chosen by paediatricians, as well as the clinical and bacteriological results. Clinical cure was achieved in 87% of patients, improvement in 9.1% and failure in 1.1% of subjects irrespective of the antibiotic treatment that they had received; no data for clinical evaluation was available for 21 patients (2.9%).

Table 5 gives details of microbiological evaluation. Clinical cure with penicillins ranged from 88.5% for amoxycillin + clavulanic acid to 89.2% for penicillin, ampicillin and amoxycillin; with oral cephalosporins the clinical cure was achieved in 81.1% of patients and ranged from 78.3% with cefaclor to 80.6% with cefixime. For macrolides, clinical cure ranged from 84.4% with rokitamycin to 90.9% with clarithromycin.

Complications of infection, such as adenitis or pharyngeal abscess, occurred in 10 cases and adverse events, usually affecting the skin and gastro-intestinal tract, in 11.

5. Discussion

The aim of antibiotic therapy in the treatment of pharyngotonsillitis should be not only clinical cure but, above all, bacteriological eradication, to avoid the rare but serious post-streptococcal sequelae.

This study has shown that erythromycin resistance rates in Italy are relatively high (23.7%) but not uniformly distributed over the national territory. The most common type of macrolide resistance in *S. pyogenes* was found to be due to the recently described efflux pump in streptococci [26] specific for 14 and 15 mem

Table 3
Clinical and bacteriological results (%) for the major groups of antibiotics used in the therapy of group A streptococcal pharyngitis

Prescribed antibiotics	Patients (*n*)	Bacteriological eradication	Clinical cure	Clinical improvement	Clinical failure	N.D.
Penicillin/amp./amoxycilllin	121	88.4	89.2	7.4	0.8	2.5
Amoxycillin + clavulanic acid	183	83.6	88.5	8.7	0.5	2.2
Oral cephalosporins	159	76.7	81.1	13.8	1.2	3.7
Clarithromycin	143	85.3	90.9	3.5	1.4	4.2
Azithromycin	75	73.3	85.3	12	2.7	0
Rokitamycin	32	59.4	84.4	12.5	0	3.1
Total	713	81	87	9.1	1.1	2.8

Table 4
Eradication of strains following treatment with macrolides-analysis on the basis of erythromycin resistance of the isolate at baseline

Antibiotics	Total strains (*n*)	Erythromycin-resistant strains		Erythromycin-sensitive strains	
		At baseline *n* (%)	Negative post-treatment *n* (%)	At baseline *n* (%)	Negative post-treatment *n* (%)
Clarithromycin	143	30 (21)	18 (60)	113 (79)	104 (92)
Azithromycin	75	16 (21.3)	6 (37.5)	59 (78.7)	49 (83)
Rokitamycin	32	9 (28.1)	6 (66.7)	23 (71.9)	13 (56.5)
Total	250	55 (22)	30 (54.5)	195 (78)	166 (85.1)

bered macrolides described as the M phenotype (41.7%). This data is similar to that obtained from other studies carried out in Italy.

Clinical cure was achieved in a high percentage of cases, irrespective of the antibiotic prescribed. The best clinical efficacy was achieved following therapy with amoxycillin and clarithromycin (90.9%) with oral cephalosporins giving 81.1% clinical cure.

Bacterial eradication was similar for clarithromycin and penicillins (85.3 and 88.4% respectively) in spite of higher resistance rates of clarithromycin. Conversely rokitamycin which had a markedly better 'in vitro' activity with respect to the other macrolides tested showed the lowest eradication rates (59.4%).

Interestingly, this study demonstrated that treatment with azithromycin was able to select resistance in strain of *S. pyogenes*. In four of 20 cases of failed eradication following azithromycin therapy it was found that the isolates had changed from being susceptible (MICs = 0.12 mg/l) to being resistant with phenotypes IR and M (MICs $4 \geq 128$ mg/l) following therapy. In contrast in clarithromycin treated cases there was found to be no change in the susceptibility pattern of strains isolated before and after therapy.

Genotyping of all strains isolated prior and after therapy also provided useful information concerning true bacterial eradication as it distinguished cases due to bacterial recolonization that would have otherwise been wrongly classified as microbiological failures. For example of the microbiological failures of cephalosporins such as cefixime (25.8%) and cefuroxime axetil (31.2%) recolonization after therapy was found to occur in 10 and 6%, respectively. In the case of macrolides however, microbiological failures were all confirmed to be due to failed bacterial eradication, as there were no cases of bacterial recolonization.

Generally some of the true failed bacterial eradication cases who had received therapy with β-lactam agents may be attributable to the presence of a recently described internalization gene[5] in group A streptococci.

The higher percentages of true failed eradication rates of amoxycillin + clavulanic acid with respect to those with amoxycillin therapy alone could either be due to the presence of the internalization gene in a higher proportion of isolates treated with the combination agent or to the poor compliance in view the poor taste of this formulation.We thank the Pediatricians and the local microbiologists who made possible the execution of this study:

Acknowledgements

LOMBARDIA. Abbiategrasso, Dr ssa A. Colombini; Melegnano, Dr ssa D. Corbella, Dr A. Gruppioni, Dr ssa G. Sturaro, Dr ssa M. Arghittu; Magenta, Dr Santucci, Dr ssa M.E. Bertoglio, Dr ssa C. Garavaglia, Dr ssa M.T. Ogliari, Dr P.M. Paganuzzi, Dr L. Pastori, Dr ssa A. Viola, Dr ssa C. Volpe, Dr ssa C. Bezzi; Mantova, Dr R. Rosignoli, Dr ssa N. Chiramonte, Dr ssa P. Dell'Anna, Dr ssa G. Diazzi, Dr ssa F. Ferrari, Dr ssa C. Orioli, Dr ssa, R. Spaggiari, Dr M. Fraizzoli, Dr F. Severi, Dr A. Raballo; Milano, Fatebenefratelli: Dr Collo, Dr ssa M. Saudelli. Pio Albergo Trivulzio: Dr ssa M. T. Lassini, Dr ssa D. Dallari, Dr ssa M. Marin, Dr S. Frugoni. Chiavenna, Dr ssa G. Valsecchi, Dr ssa Cesabianchi, Dr Lauretta, Dr ssa M. R. Dell'acqua; Casorate Primo, Dr P. Buratti; Sondalo: Dr ssa M. Biancotti, Dr ssa C. Pancrazi, Dr ssa P. Troupioti; Manerbio, Dr ssa Romanini, Dr ssa Derelli, Dr G. Tettoni; Lecco–Merate–Bellano, Dr ssa V. Mazzoleni, Dr B. Spinelli, Dr ssa M. Micheli, Dr Bosisio Parini, Dr ssa C. Anghileri, Dr ssa M.R. Baratto, Dr ssa T. D'Orazio, Dr ssa M. Colombo, Dr ssa M. Petrone, Dr ssa A. Villella, Dr ssa S. Gabiazzi, Dr L. Gatto, Dr C. Marcolini, Dr A. Sala. PIEMONTE. Alba-Bra, Professor S. Cocuzza, Dr F. Spinelli, Dr ssa P. Giacotto, Dr ssa A. Santoro, Dr G. Di Francesco, Dr ssa L. Piazza, Dr G. Ponzo, Dr G. Mauro, Dr M. Toppino; Alessandria, Dr ssa S. Camilli, Dr S. Gandini, Dr A. Lo Faro, Dr F. Allegranza, Dr A. La Mantia, Dr Cattaneo, Dr G. Comazzi, Dr ssa F. Milano; Pinerolo, Dr ssa Panfani, Dr Picotto, Professor ssa D. Savoia; Vercelli, Dr ssa M. Colombo, Dr L. Barberis, Dr E. Vercelloni, Dr M. Ferri, Dr B. De Maestri, Dr Guazzotti. VENETO: Bassano del Grappa, Dr ssa M. Finco, Dr ssa R. Scala, Dr ssa P. Bonin, Dr ssa M. Marcadella, Dr ssa T. Centoro, Dr M.

Table 5
Bacterial eradication versus recolonization on the basis of genotype identification of *S. pyogenes* strains isolated at baseline and follow-up

Prescribed antibiotics	Patients with follow-up after treatment (*n*)	Not eradicated *n* (%)	Colonisation by new strain *n* (%)
Penicillins including ampicillin, amoxycillin	121	12 (85.7)	2(14.3)
Amoxycillin + clavulanic acid	183	25 (83.3)	5 (16.7)
Cefaclor	92	18 (100)	0
Cefixime	31	5 (62.5)	3 (37.5)
Cefuroxime axetil	16	4 (80)	1 (20)
Ceftibuten	20	6 (100)	0
Azithromycin	75	20 (100)	0
Clarithromycin	143	21 (100)	0
Rokitamycin	32	13 (100)	0
Total	713	124 (91.9)	11 (8.1)

Menara, Dr N. Grassi, Dr W. Tesser. EMILIA ROMAGNA. Bologna, Professor G. Salvioli, Dr ssa P. Stancari, Dr ssa A. Virgili, Dr ssa E. Magnaterra, Dr P. Calzolari, Dr ssa P. Rampin, Dr ssa A. Licigniano, Dr ssa R. A. Palumbo, Dr ssa O. Parisini, Dr ssa L. Felicani, Dr ssa T. Fornasari, Dr ssa C. Alonge; Imola, Dr L. Reggiani, Dr ssa D. Bertocchi, Dr ssa N. Cevenini, Dr P.L. Cremonini, Dr G.B. Figna, Dr ssa C. Cassani. CAMPANIA. Napoli, Professor A. F. Capristo, Dr F. Decimo, Dr ssa N. Maiello, Dr M. Miraglia del Giudice, Dr F. P. Brunese, Dr L. Cioffi, Professor ssa M.A Tufano; Cava dei Tirreni, Dr ssa I. Andreozzi, Dr D. Viggiano, Dr M. Salsano, Dr B. Salomone, Dr L. Pisani, Dr ssa G. D'Adamo, Dr ssa P Sabatini; Salerno, Dr E. Caliendo, Dr G. Basilio, Dr ssa G. Mussari, Dr A. Di Giglio, Dr F. Mandia, Dr ssa L. Capuano. CALABRIA. Catanzaro, Professor A. Focà, Dr L. Ranieri, Dr ssa A. Gullo, Dr S. Grotteria. SICILIA. Catania, Professor ssa G. Tempera, Dr G. Bottaro, Dr ssa A. Nicosia, Dr ssa R. Papa, Dr ssa M. Rutella, Dr S. Bonforte, Dr R. Bucchieri, Dr R. Corallo, Dr P. De Luca, Dr G. Ficarra, Dr ssa G. Cosentino, Dr A. Gulino, Dr G. Mazzola, Dr P. Melillo, Dr F. Prestifilippo, Dr F. Caccamo.

References

[1] Komaroff AL, Aronson MD, Pass TM, et al. Serologic evidence in chlamydial and mycoplasmal pharyngitis in adult. Science 1983;222:927–9.
[2] Radetsky M. Group A streptococcal infections. In: Gellis and Kagan, editors. Current Paediatric Therapy. 14th ed. Philadelphia, PA: Saunders, 1993. p. 557.
[3] Quinn RW. Epidemiology of group A streptococci infections — the changing frequency and severity. Yale J Biol Med 1982;55:265–70.
[4] Phillips G, Parratt D, Orange GV, et al. Erythromycin-resistant *Streptococcus pyogenes*. J Antimicrob Chemother 1990;25:723–4.
[5] Gnomon R, Keller N, Brasilia A, et al. Prevalence of internalization-associated gene, prF1, among persisting group A streptococci strains isolated from asymptotic carriers. Lancet 1998;352:1974–7.
[6] 'Mycoplasma pneumoniae' Editorial Lancet 1991; 337: 651–2.
[7] Breese BB, Hall CB. Beta-hemolytic streptococcal disease. Boston, MA: Houghton Mifflin, 1978.
[8] Dillon HC. Streptococcal pharyngitis in the 1980s. Pediatr Infect Dis 1987;6:123–30.
[9] Duval J. Evolution and epidemiology of MLS resistance. J Antimicrob Chemother 1985;16:137–49.
[10] Kliegman RM, Feigin RD. Streptococcal Infections. Nelson-Text book of Paediatrics, 14th ed. Philadelphia, PA: Saunders, 1992. p. 698.
[11] Stingemore N, Francis GR, Toohey M, et al. The emergence of erythromycin resistance in *Streptococcus pyogenes* in Fremantle, Western Australia. Med J Australia 1989;150:626–31.
[12] Seppala H, Nissinen A, Jarvinen H, et al. Resistance to erythromycin in group A streptococci. N Engl J Med 1992;326:292–7.
[13] Zackisson G, Lind L, Roos K, et al. Erythromycin-resistant beta-haemolytic streptococci group A in Goteborg, Sweden. Scand J Infect Dis 1988;20:419–20.
[14] Jarvinen HA, Nissinen A, Huovinen P. Erythromycin resistance in Group A streptococci. Lancet 1989;1:1022–3.
[15] Maruyama SH, Yoshioka H, Fujita K, et al. Susceptibility of Group A streptococci to antibiotics: prevalence of resistance to erythromycin in Japan. Am J Child 1979;133:1143–5.
[16] Miyamoto Y, Takizawa K, Matsushima A, et al. Stepwise acquisition of multiple drug resistance by beta-hemolytic streptococci and differences in resistance pattern by type. Antimicrob Ag Chemother 1978;13:399–404.
[17] Hsuech PR, Chen HM, Huang AY, et al. Decreased activity of erythromycin against *Streptococcus pyogenes* in Taiwan. Antimicrob Ag Chemother 1995;39:2239–42.
[18] Borzani M, Varotto F, Garlaschi L, et al. Clinical and microbiological evaluation of miocamycin activity against group A streptococci in paediatric patients: 'three years' incidence of erythromycin resistant Group A streptococci. J Chemother 1989;1:35–8.
[19] Cellesi C, Chigiotti S, Zanchi A, et al. Susceptibility to macrolide and beta-lactam antibiotics of *Streptococcus pyogenes* strains isolated over a four year period in central Italy. J Chemother 1996;8:188–92.
[20] Cocuzza CE, Mattina R, Mazzariol A, et al. High incidence of erythromycin-resistant *Streptococcus pyogenes* in Monza (North Italy) in untreated children with symptoms of acute pharyngotonsillitis: an epidemiological and molecular study. Microbial Drug Resistance 1997;3(4):381–8.

[21] Scopetti F, Pataracchia M, Pacifico L, et al. A multicenter study on antimicrobial susceptibility and typing of group A streptococci from cases of pharyngotonsillitis and scarlet fever. Microecol Ther 1995;25:348–55.
[22] Cornaglia G, Ligozzi M, Mazzariol A, et al. Rapid increase of resistance to erythromycin and clindamycin in *Streptococcus pyogenes* in Italy, 1993–1995. Emerging Infect Dis 1993;2:331–4.
[23] Pavesio D, Nicoletti G, Ripa S, et al. Progetto Artemis Italia: aspetti microbiologici e clinici nelle faringotonsilliti da *Streptococcus pyogenes* in età paediatrica. GIMMOC 1999;3:5–32.
[24] Pavesio D, Nicoletti G, Ripa S, et al. Secondo Progetto Artemis Italia: aspetti microbiologici e clinici nelle faringotonsilliti acute da *Streptococcus pyogenes* in età paediatrica. GIMMOC 1999;3:3–41.
[25] Seppala H, Nissinen A, Jarvinen H, et al. Three different phenotypes of erythromycin-resistant *Streptococcus pyogenes* in Finland. J Antimicrob Chemother 1993;32:885–91.
[26] Sutcliffe J, Tait-Kamradt A, Wondrack L. Streptococcus pneumoniae and *Streptococcus pyogenes* resistant to macrolides but susceptible to Clindamycin:a common resistance pattern mediated by an efflux system. Antimicrob Ag Chemother 1996;40(8):1817–24.
[27] Breese BB. A simple scorecard for the tentative diagnosis of streptococcal pharyngitis. Am J Dis Child 1977;135:514–7.
[28] National Committee for Clinical Laboratory Standards. Performance Standards for Antimicrobial Susceptibility testing. Fifth Informational Supplement-NCCLS Document M 100 S6, vol. 15, no. 14, 1995.
[29] National Committee for Clinical Laboratory Standard. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. M7-A3, National committee for Clinical Laboratory Standatds, Villanova, PA, 1995.
[30] Tenover FC, Arbeit RD, Goering RV, et al. Interpreting chromosomal DNA restriction patterns procedure by pulsed-field gel electrophoresis: Criteria for bacterial typing. J Clin Microbiol 1995;33:2233–9.