Bacteriological aspects of treatment failures in streptococcal tonsillitis

by

Eva Grahn

Umeå University 1986
Illustration on cover
"Streptococcal tonsillitis"
Painting by the author
Bacteriological aspects of treatment failures in streptococcal tonsillitis

AKADEMISK AVHANDLING
som med vederbörligt tillstånd av
Medicinska fakulteten vid Umeå Universitet
för avläggande av Dr med sci
kommer att offentligen försvaras i lasarettets
byggnad 1 D, sal B, 9 trappor
fredagen den 26 september 1986, kl. 09.00

av

Eva Grahn
ABSTRACT

ß-hemolytic streptococci persist in 10-25% of patients with acute streptococcal tonsillitis (about 10,000-25,000 per year in Sweden) in spite of treatment with a recommended dosage and schedule of phenoxyethylpenicillin. The aim of the study was to investigate different bacteriological factors involved in treatment failures of streptococcal tonsillitis. Patients included in the study were 35 patients who underwent tonsillectomy, 62 persons included in a tonsillitis epidemic outbreak, 267 tonsillitis patients contacting the ENT-clinic, Sahlgrenska Hospital, Göteborg, and 20 healthy volunteers taking phenoxyethylpenicillin. It was found that the Steer's steel pin replicator was a useful tool to study interference between α- and ß-hemolytic streptococci and a quantitative difference in the inhibitory capacity of the different α-strains was noted. α-streptococci with a strong inhibitory capacity on β-streptococci were isolated mainly from individuals seemingly resistant to β-streptococcal tonsillitis, while from patients with repeated tonsillitis no or low numbers of inhibiting α-streptococci were demonstrated. Patients with clinical treatment failure had less α-streptococci with inhibiting capacity on their own β-streptococcal strain compared with the healthy carriers. These treatment failures also showed beta-lactamase activity in their saliva pellet significantly more often than patients in the control groups. In volunteers penicillin was released from ordinary sugar coated tablets already in the mouth resulting in a decrease of the α-streptococcal flora. A synergistic effect on β-hemolytic killing by low concentration of penicillin and inhibition of α-streptococci was noted in vitro and in vivo. Penicillin tolerance was registered in most strains from the treatment failure group, but in none of the strains from the group of successfully treated patients. A co-operation between different bacteriological factors (bacterial interference, beta-lactamase production, penicillin tolerance) seems to be important in treatment failures of streptococcal tonsillitis.

Key words: ß-streptococci, treatment failure, α-streptococci, bacterial interference, beta-lactamase, phenoxyethylpenicillin, penicillin tolerance
Bacteriological aspects of treatment failures in streptococcal tonsillitis

by

Eva Grahn

Umeå University 1986
To my homevillage Ale
Bacteriological aspects of treatment failures in streptococcal tonsillitis

Eva Grahn, Department of Clinical Bacteriology, University of Umeå, Umeå, Sweden

ABSTRACT

β-hemolytic streptococci persist in 10-25% of patients with acute streptococcal tonsillitis (about 10,000-25,000 per year in Sweden) in spite of treatment with a recommended dosage and schedule of phenoxyethylpenicillin. The aim of the study was to investigate different bacteriological factors involved in treatment failures of streptococcal tonsillitis. Patients included in the study were 33 patients who underwent tonsillectomy, 62 persons included in a tonsillitis epidemic outbreak, 267 tonsillitis patients contacting the ENT-clinic, Sahlgrenska Hospital, Göteborg, and 20 healthy volunteers taking phenoxyethylpenicillin. It was found that the Steer's steel pin replicator was a useful tool to study interference between α- and β-hemolytic streptococci and a quantitative difference in the inhibitory capacity of the different α-strains was noted. α-streptococci with a strong inhibitory capacity on β-streptococci were isolated mainly from individuals seemingly resistant to β-streptococcal tonsillitis, while from patients with repeated tonsillitis no or low numbers of inhibiting α-streptococci were demonstrated. Patients with clinical treatment failure had less α-streptococci with inhibiting capacity on their own β-streptococcal strain compared with the healthy carriers. These treatment failures also showed beta-lactamase activity in their saliva pellet significantly more often than patients in the control groups. In volunteers penicillin was released from ordinary sugar coated tablets already in the mouth resulting in a decrease of the α-streptococcal flora. A synergistic effect on β-hemolytic killing by low concentration of penicillin and inhibition of α-streptococci was noted in vitro and in vivo. Penicillin tolerance was registered in most strains from the treatment failure group, but in none of the strains from the group of successfully treated patients. A co-operation between different bacteriological factors (bacterial interference, beta-lactamase production, penicillin tolerance) seems to be important in treatment failures of streptococcal tonsillitis.

Key words: β-streptococci, treatment failure, α-streptococci, bacterial interference, beta-lactamase, phenoxyethylpenicillin, penicillin tolerance
This thesis is based on the following original papers referred to in the text by their Roman numerals

I Interference of α-hemolytic streptococci isolated from tonsillar surface on β-hemolytic streptococci (Streptococcus pyogenes) - A methodological study Grahn, E., Holm, SE., Ekedahl, C. and Roos, K.

II Bacterial interference in the throat flora during a streptococcal tonsillitis outbreak in an apartment house area. Grahn, E. and Holm SE.

III Evaluation of beta-lactamase activity and microbial interference in treatment failures of acute streptococcal tonsillitis.
Roos, K., Grahn, E. and Holm SE.
Accepted for publication in Scand.J.Infect.Dis.

IV Penicillin concentration in saliva and its influence on bacterial interference. Grahn, E., and Holm, SE.
Accepted for publication in Scand.J.Infect.Dis.

V The effect of penicillin on bacterial interference in vivo.
Grahn, E. and Holm, SE.
Accepted for publication in Scand.J.Infect.Dis.

VI Penicillin tolerance in β-streptococci isolated from patients with streptococcal tonsillitis. Grahn, E., Holm, SE. and Roos, K.
Accepted for publication in Scand.J.Infect.Dis.
CONTENTS

ABBREVIATIONS

DEFINITIONS

INTRODUCTION .............................................................................................................. 9
  Treatment failure in streptococcal tonsillitis .......................... 9
  Penicillin in the treatment of streptococcal tonsillitis .. 9
  Beta-lactamase and treatment failures ................................. 11
  Bacterial interference ............................................................... 13
  Sensitivity of ß-streptococci to antibiotics ............................ 15

AIMS OF THE STUDY .................................................................................................. 17

MATERIAL AND METHODS ........................................................................................... 18

RESULTS AND DISCUSSION ...................................................................................... 26
  Methodological and epidemiological aspects on bacterial
  interference ................................................................................................. 26
  Bacterial interference in treatment failures ........................... 29
  Beta-lactamase activity in saliva ................................................. 30
  Penicillin concentration in saliva and serum ......................... 31
  Influence of penicillin on bacterial interference in
  vitro ........................................................................................................ 33
  The effect of penicillin on bacterial interference in
  vivo ........................................................................................................ 35
  Penicillin tolerance and sensitivity of ß-streptococci
  to other antibiotics ................................................................. 36

GENERAL SUMMERY ....................................................................................................... 39

GENERAL REMARKS AND CONCLUSION .................................................................... 42

ACKNOWLEDGEMENTS ................................................................................................ 44

REFERENCES .................................................................................................................. 45

Paper I-VI
ABBREVIATIONS

GAS - group A streptococci

CFU - colony forming units

PC-V - phenoxymethylpenicillin

PC-G - benzylpenicillin

MIC - minimal inhibitory concentration

MBC - minimal bactericidal concentration

I.M. - intramuscularly

Tcf - tissue cage fluid

DEFINITIONS

Acute streptococcal tonsillitis was defined as an acute inflammatory reaction of the tonsils caused by β-streptococci.

"Clinical treatment failure" was defined as infection with the same type of streptococcus as the one isolated during the preceding infection within two weeks after therapy.

"Bacterial treatment failure" refers to cases in which the original type of streptococcus was isolated within two weeks after therapy, but without clinical signs of tonsillitis.

"Resistant to streptococcal tonsillitis" persons not infected with β-streptococci in spite of close contact during a long time (6 months) to family members with β-streptococcal tonsillitis.
"Mixed saliva" consisted of parotid, submandibular, sublingual and minor gland saliva, oral bacteria, desquamated epithelial cells and gingival fluids.

"Saliva supernate", the supernate obtained after centrifugation of mixed saliva.

"Saliva sediment", the sediment obtained after centrifugation of mixed saliva.

Penicillin tolerance was defined as a MBC/MIC ratio >16.

Paradoxical effect was defined as increasing numbers of survivors at penicillin concentrations higher than MBC.
INTRODUCTION

Treatment failure in streptococcal tonsillitis:
It has been estimated that at least 300,000 persons in Sweden yearly suffer from acute tonsillitis. About 30-50% is caused by β-streptococci and since treatment failures are common this condition creates therapeutic as well as economical consequences. The high treatment failure rate 10-25% (1-5) in β-streptococcal tonsillitis is a multifaceted problem. Besides local deficiency in the host's defence and general immunological defects (6), inadequate antibiotic activity at the site of infection has been mentioned. The latter includes patient compliance, dosage of penicillin, number of doses and impaired absorption. Also inactivation of penicillin by beta-lactamase producing bacteria resulting in an insufficient penicillin concentration at the focus of infection has been mentioned (8). Other factors which have been connected with treatment failures in streptococcal tonsillitis are disturbance in the normal flora resulting in an increased susceptibility to streptococcal infections (8,9) and penicillin tolerance in β-streptococci group A (10).

Penicillin in the treatment of streptococcal tonsillitis.
In studies concerning patient compliance Charney et al (11) found that 81% of the patients were taking the penicillin as prescribed on the fifth day, and 56% still on the ninth day. The treatment failure rate of patients with acute pharyngitis could by Green et al (12) be correlated with drug defaulting. Roos et al (5) showed that 39% of the patients had failed to take at least one dose of pc-V during a 10 days' treatment
period, but all patients with clinical treatment failure had taken all their tablets.

The recommended dosage of phenoxybenzyl-penicillin in Sweden is 12.5 mg/kg body weight, twice a day. In the study of Roos et al (5) this dose was given as well as 25 mg/kg of pc-V to some patients. The higher dose did not correlate to a lower rate of treatment failure. The number of doses has been reduced to two doses daily (13, 14) on the recommendation by the Swedish National Board of Health and Welfare 1982. The duration of therapy is still ten days for streptococcal tonsillitis, although attempts have been made to reduce the time to seven days with penicillin or five days with clindamycin (15, 16). However, higher treatment failure rates have been noted in studies with 5 or 7 days of treatment, compared to 10 days (15, 16).

Pharmacokinetic studies in serum and tonsillar tissue (17-19) have confirmed that adequate penicillin levels are reached using the recommended dosage. In our study (18) we reported on a concentration above MIC (0.03 mg/l) for most β-hemolytic streptococci group A during at least 4-5 hours in tonsillar tissue. The antibiotic concentration attained in saliva of healthy individuals has been investigated by several authors (20-23). Thus, Speirs et al (20) registered a penicillin concentration of <0.03-3.25 mg/l 30 minutes after administration in mixed saliva, but no activity was detectable when penicillin from the same batch was given in capsules. In the study of McCracken et al (22) the patients were given penicillin suspension resulting in a high and longlasting penicillin con-
centration in saliva. The significance of salivary concentration of penicillin for the cure rate has not been thoroughly investigated, but Sukchotiratana et al (23) demonstrated that ordinary doses of penicillin and clindamycin change the total bacterial flora within 12 hours after a single dose. Several studies have also been carried through to compare the efficiency of different antibiotics in relation to cure rate (3, 15, 24-26). Thus, Breese et al (26), Randoph and Dettaan (24), Howie and Plousard (3) reported on a higher failure rate of streptococcal tonsillitis or carriers with orally given penicillin compared with lincomycin. Also Sinanian et al (15) could show a higher failure rate with phenoxymethyl penicillin than with clindamycin. Comparing penicillin with cefadroxil and erythromycin the highest failure rate was found when using penicillin, although there were no significant differences (25).

Beta-lactamase and treatment failures.
The role of beta-lactamase producing bacteria for the persistence of beta-streptococci after penicillin treatment of acute tonsillitis has been discussed. Thus, Brees et al (26) found fewer beta-streptococcal carriers after treatment with penicillinase-stable antibiotics compared with penicillin V, indicating that penicillinase production could protect the beta-streptococci from the action of penicillin. In this context it should be recalled that the administration of penicillin can change the normal flora and allow the emergence of beta-lactamase producing organisms (35) that can also spread to other household contacts. Several authors (27,28) suggested that penicillinase producing Staphylococcus aureus in the throat
flora inactivated the penicillin and were more often found in patients with treatment failure. However, Quie et al (29) could not confirm this finding. Also other penicillinase producing bacteria have been incriminated in connection with treatment failure in streptococcal tonsillitis, especially Bacteroides species and Fusobacteria (30, 31). Brook et al (32) investigated the aerobic as well as the anaerobic flora in tonsils of children with recurrent tonsillitis and found that 74% of the patients had beta-lactamase producing organisms. In a study of Turnér and Nord (31), 73% of the patients with recurrent tonsillitis were found to be colonized with beta-lactamase producing bacteria. Brook et al (33) showed how Bacteroides fragilis protected group A streptococci from the action of penicillin. This phenomenon could also be demonstrated by Scheifele and Fussell (34) when beta-lactamase producing Haemophilus parainfluenzae was mixed with ampicillin and group A streptococci.

The most common way to study the role of beta-lactamase production in the throat is to isolate the bacteria and test the isolates for beta-lactamase production. A discrepancy between in vivo efficacy and in vitro activity can therefore not be ruled out. Brook et al (36) investigated the beta-lactamase activity in tonsillar tissue from children with recurrent tonsillitis and correlated these findings with the presence of aerobic and anaerobic beta-lactamase producing strains of these tonsils. Beta-lactamase activity was found in 85% of the 39 tonsils that harbored beta-lactamase producing bacteria. In a recent study by Turnér and Nord (not published) beta-lactamase activity was observed in saliva in volunteers treated with
phenoxymethylpenicillin and a significant increase in the 
beta-lactamase activity parallel to the increase of beta-lact-
amase producing bacteria was noted.

Bacterial interference

Bacterial interference may play an important role for the pro-	ection against tonsillitis as well as against renewed infec-
tions. Several factors are involved in the interference bet-
ween microorganisms, such as pH, accumulation of waste pro-
ducts, (like ammonia, lactic acid, free fatty acids) competi-
tion for essential growth substances, bacteriophages, peroxide 
production, bacterial toxins, enzymes and bacteriocins (37).

Sanders (38) investigated the interference among bacteria in 
the flora of the respiratory tract and found that viridans 
streptococci inhibited the growth of group A streptococci as a 
result from induction of an acidic medium and depletion of 
esential substrate factors. Crowe (9) and Sanders (39) did 
further studies on the influence of the normal flora on group 
A streptococci and could demonstrate that children who did not 
get colonized with group A streptococci had a significantly 
greater inhibitory activity of their α-streptococci than 
children who often had group A infections. It was seen that 
the number of inhibiting α-streptococci was increasing after 
group A infections and they also pointed out that the inhibi-
ting activity increased with age. Sanders et al (8) demonstra-
ted that oral treatment of penicillin in generally recommended 
doses resulted in a decrease of inhibiting bacteria in the 
oral flora which persisted three weeks after therapy. Further-
more, Sprunt et al (40) demonstrated an elimination of α-st-
reptococci from the throat during intensive antibiotic treat-
ment resulting in an overgrowth of Enterobacteriaceae spp. Beck (41) observed a high interfering activity of a Streptococcus viridans strain isolated from a person seemingly resistant to infection with β-hemolytic streptococci. This strain inhibited completely 72% and partially 28% of the group A streptococci tested. He suggested an "implantation" with this strain to patients who suffer from frequent or recurrent infections of group A streptococci. One year later Sprunt et al (42) implanted an α-streptococcal strain in nasopharynx of 22 infants that were considered to be a high risk of infection because of abnormal pharyngeal colonization with potential pathogens. In 16 of the infants the implantation was successful and α-streptococci were the predominant pharyngeal flora within 48 to 72 hours. The implantation was clearly responsible for conversion of the flora to normal in 7 infants in that the implanted strain was the only strain of α-streptococcus recovered from 4-18 days after implantation. However, in other children the implantation initiates natural selective mechanisms of pharyngeal mucosa for colonization by other α-streptococci.

As earlier mentioned several factors may influence upon the interference between microorganisms. The mechanism of interference in vivo is not yet defined but many strains of α-hemolytic streptococci produce bacteriocin-like substances. One of them, Viridin B has been partially purified and characterized by Dajani and coworkers (43, 44). Later on, Sanders and Sanders (45) reported on an antibiotic produced by Streptococcus salivarius, inhibitory to group A streptococci. This was recovered from cell-free filtrates at the end of the loga-
rhythmic growth phase, in contrast to the viridins that are obtained by mechanical disruption of the bacterial cell. Furthermore, Deepika and Slade (46) demonstrated a bacteriocin from Streptococcus mutans bactericidal for group A streptococci. This substance was produced in the growth medium during the stationary phase of growth.

In contrast also β-streptococci can produce a bacteriocin, as shown by Tagg et al (47). This bacteriocin of group A streptococci, streptococcin A-FF22, is produced by an L-form strain FF22, is plasmid controlled and can be transduced (48). Kolenichenko and Totolyan (49) reported that group A streptococci producing a bacteriocin-like substance dominate during a period of high incidence of acute streptococcal disease. Since this bacteriocin deeply influence upon the ordinary oral bacterial flora, streptococcin A-FF22 might be considered as a virulence factor of potential clinical significance in certain epidemiological situations.

Sensitivity of β-streptococci to antibiotics

Penicillin resistance in beta-streptococci group A has never been observed over the years. But resistant mutants have been isolated in the laboratory (50). The MIC for most β-hemolytic streptococci group A is <0.03 mg/ml. However, higher figures have been reported for some group C and G strains (51). Resistance to other antibiotics has been reported. In 1972 Dixon and Lipinski (52) reported of a 0.05% resistance of
β-streptococci group A to lincomycin and erythromycin. Among group A streptococci isolated during 1974 to 1975 in Japan, 60% of the strains were resistance to erythromycin and 70% to tetracycline (53). Also in Sweden observations of resistance to erythromycin and tetracycline have been reported (54, 55). A new type of decreased penicillin sensitivity in Staphylococcus aureus was reported by Sabath et al. (56). Later on, this phenomenon was given the name penicillin tolerance and was defined as an increased MBC/MIC ratio (>16 ->100). Penicillin tolerance has been found in group B (57), group C (58), group D and G (59, 60) and group A streptococci (61, 62). Whether penicillin tolerance has a clinical significance or not has been debated. However, Pulliam (63) showed that in rabbits with experimental endocarditis induced by tolerant viridans streptococci, bacteria could still be recovered after 5 days of penicillin treatment. Similar findings have been done in patients with endocarditis infected with tolerant staphylococci resulting in treatment failures (64).
AIMS OF THE STUDY

Studies on factors of significance for treatment failure rate in streptococcal tonsillitis have usually been focused on single clinical or bacteriological parameters. The present study gives a diversified general picture of various bacteriological factors that lately have been debated as possible factors that may influence on the treatment failure rate.

The aims of this study were:

-to develop suitable methods to test interference between α- and β-hemolytic streptococci (I)

-to examine the role of the normal throat flora for resistance to streptococcal tonsillitis (II, III).

-to examine the role of beta-lactamase in saliva in patients with clinical treatment failure (III).

-to analyse the kinetics of phenoxymerhylpenicillin in saliva and to illustrate how penicillin in various concentrations can influence on the interference between α- and β-streptococcal in vitro (IV) and in vivo (V).

-to elucidate the possible significance of penicillin tolerance for treatment failure in patients with streptococcal tonsillitis (VI).
MATERIAL AND METHODS

Patient material

In paper I, 33 patients who underwent tonsillectomy due to peritonsillar abscesses or recidivating tonsillitis were included in the study. Another patient material consisted of 23 adults and 22 children living in apartments in 3 buildings, where an epidemic outbreak of GAS tonsillitis occurred (paper II). Tonsillitis patients contacting the ENT-clinic, Sahlgrenska Hospital, Göteborg were included in the next two studies (paper III, VI). One hundred and sixty-nine of the 267 patients had a positive culture for beta-hemolytic streptococci. Out of these 169 patients 132 were cured after treatment. Nineteen (11%) suffered a recurrence infection within 2 weeks of completion of treatment. Thirteen (8%) had the same strain (clinical treatment failure) while another strain than the original one was isolated from 6 (3%). 24 patients (14%) harboured the same streptococcal strain (bacterial treatment failure) but were clinically healthy. Thus, in all 22% (13 + 24 patients) were regarded as treatment failures. 20 healthy volunteers were included in paper IV.

Bacterial strains

Several bacterial strains are used in these six papers and they are all listed in Table I. 52 alpha-hemolytic streptococci isolated from 33 patients who had undergone tonsillectomy were used in paper I. The indicator strains of beta-hemolytic strep-
tococci used were different types of group A streptococci from Dr Lancefield's collection and also clinical isolates of type T12 and T49 (paper I). β- and α-hemolytic streptococci isolated from patients in an epidemic outbreak of GAS tonsillitis were used in paper II. In paper III β- and α-hemolytic streptococci were isolated from 13 patients with clinical treatment failure and from 12 healthy contacts. In the in vitro study the following strains were used: Streptococcus mitis (a1), Streptococcus sanguis (a2155) isolated from persons with tonsillitis and Streptococcus sanguis (a29) isolated from a person seemingly resistant to tonsillitis in a tonsillitis prone family. The β-streptococci used were 4 different reference strains (T-types 4, 6, 12, 49) and a clinical isolate (T4 clin) (paper IV). Some of these strains were also used in the in vivo study, paper V. In the last paper, (paper VI), β-streptococci from patients with clinical treatment failure and patients successfully cured with penicillin were used.

**Animals**

Eight white New-Zealand rabbits were used in paper I and nine rabbits from improved breed of French hydraulic ram/chinchilla were used in paper V.
Table I

Bacterial strains:

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Isolated from</th>
<th>In paper</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-streptococci</td>
<td>different T-types of GAS from dr Lancefield's collection</td>
<td>I,II,III,V</td>
</tr>
<tr>
<td>β-streptococci</td>
<td>different epidemics in Europe, kindly supplied by dr. Poul Christensen, Lund</td>
<td>I</td>
</tr>
<tr>
<td>β-streptococci</td>
<td>23 adults and 22 children during an epidemic outbreak of GAS tonsillitis</td>
<td>II</td>
</tr>
<tr>
<td>β-streptococci</td>
<td>13 patients with clinical treatment failure and their healthy contacts</td>
<td>III</td>
</tr>
<tr>
<td>GAS T4 clin</td>
<td>a tonsillitis patient</td>
<td>IV,V</td>
</tr>
<tr>
<td>β-streptococci</td>
<td>tonsillitis patients successfully treated with penicillin</td>
<td>VI</td>
</tr>
<tr>
<td>α-streptococci</td>
<td>33 patients with recidivating tonsillitis</td>
<td>I,IV,V</td>
</tr>
<tr>
<td>α-streptococci</td>
<td>23 adults and 22 children during an epidemic outbreak of GAS tonsillitis</td>
<td>II,IV</td>
</tr>
<tr>
<td>α-streptococci</td>
<td>13 patients with clinical treatment failure and their healthy contacts</td>
<td>III,VI</td>
</tr>
<tr>
<td>Streptococcus sanguis a(2155)</td>
<td>a tonsillitis patient</td>
<td>IV,V</td>
</tr>
</tbody>
</table>
Methods to determine bacterial interference.

All strains were grown in TY-medium (65) before used in either of the methods.

Interference on agar plates. Briefly, minidrops of the α-hemolytic strains (containing 10-100 cfu) were transferred to blood agar plates by a Steers' steel pin replicator (66) and allowed to dry. A similar sample of β-hemolytic strain was applied adjacent to each of the α-hemolytic strains and the plates were incubated in 5% CO₂ at 37°C, (Figure 1 and 2). This method was used in paper I, II, III.

Fig. 1
Interference on agar plates.
a2155 inhibiting different types of GAS

Fig. 2
Interference on agar plates.
a2155 inhibiting GAS T3
Interference in broth. Equal numbers of \( \alpha \)- and \( \beta \)-streptococci \((10^4 - 10^5 \text{cfu/ml})\) were inoculated simultaneously in TY-medium (65) in the presence or absence of penicillin and incubated at 37°C. Samples were drawn at different times and transferred to blood agar plates for viable counting (cfu). Penicillin activity in the samples were inactivated by penicillinase (Penase, 1000u/ml) in the blood agar plates (paper I and IV).

"Agar overlay plates". The \( \alpha \)-streptococci \((10^5 \text{cfu/ml})\) were spread on a blood agar plate and incubated overnight at 37°C. A second blood agar layer was poured on the top of the \( \alpha \)-streptococcal layer. After gelification \( \beta \)-streptococcal strains were stamped on the surface using Steers' steel pin replicator. The growth results were recorded after further incubation for 18 hours at 37°C (Paper I).

Interference in vivo. The tissue cage model has been described earlier (67) and the contents of the tissue cage fluid analysed (68). Briefly, four steel net chambers were implanted subcutaneously in the rabbits. Four weeks later the chambers had been covered with a layer of connective tissue and filled with a slightly yellow fluid - the tissue cage fluid. In two of the cages the \( \alpha \)- and \( \beta \)-streptococci \((10^5 \text{cfu/ml})\) were injected separately, while in the other two a mixture of these strains were injected simultaneously into each of the cages (Paper I and V).

Bacteriological procedure
Samples from the throat were taken with a dry sterile cotton swab rubbed once up and down the surface of both tonsils (III, VI) and also along the soft palatina rim (II, IV). All
swabs were immediately placed in 1 ml TY-broth (65) and shaken for 20-30 seconds. Ten ml of the suspended material and dilutions thereof (10^{-2}, 10^{-3}) were evenly distributed on blood agar plates. The plates were incubated at 37°C in 5% CO₂ overnight and the cultures examined.

The samples from all patients in paper II and IV were taken by the same person (EG) and also the ones in paper III and VI (KR). The identification of β-hemolytic streptococci were done by coagglutination technique with specific antisera (69). Typing was performed using streptococcal T-typing sera (Institute of Sera and Vaccines, Prague) and the SOR test performed following the description of Maxted and Widdowson (70). The α-hemolytic streptococci were identified by Carlssons scheme (71) (paper I and II) and by Facklam classification system (72) (paper IV, V).

**Antibiotics**

The antibiotic used for treatment of the various patient groups were regular phenoxy-methylpenicillin tablets (Calcipen, LEO, Sweden). In addition to ordinary tablets extra sugar coated tablets were used in paper IV. In the in vitro experiments of bacterial interference (paper IV) phenoxy-methylpenicillin were used. The animals in paper V were given benzylpenicillin (ASTRA, Sweden) i.m. For determination of MIC and MBC in paper VI the following antibiotics were used, phenoxy-methylpenicillin (LEO), clindamycin (Upjohn), cephadroxil (Bristol), doxycyklin (Pfizer) and erythromycin (Abbott).
Determination of penicillin in saliva and serum

All samples were tested using the agar well method (73) with TG-agar (Bacto-agar, Difco with yeast extract, Trypticase peptone, BBL and 0.2% glucose) and Bacillus Stearothermophilus (ATCC 3032) as an indicator strain (paper IV, V). The plates (14 cm Ø) were prepared by pouring 30 ml of the agar-bacterium mixture. Six mm Ø wells were punched in the agar and filled with 30 μl of the saliva samples. The plates were incubated at 56°C over night, after 30 minutes in room temperature. The sensitivity limit of the method was 0.01 mg/l. Samples as well as standards were tested in triplicates. In a similar way, but using 2.5 mm wells and 10 μl sample, the penicillin levels in serum were determined. Standard for saliva was prepared in NaCl, for serum in normal serum and for t.c.f. in 50% rabbit serum.

Beta-lactamase analysis. The presence of beta-lactamase in saliva was tested by analysis of the sediment and the saliva supernate obtained after centrifugation using chromogenic cephalosporin (87/312, Nitrocefin, Glaxo) according to the method of O'Callaghan (74) (paper III).

MIC

The MIC determination was made in TY-broth with serial doubling dilutions of antibiotics and a final inoculum of 2×10⁵-5×10⁵ cfu/ml. The MIC was determined by visual examination for turbidity (paper IV, V, VI).
MBC

After incubation 100 µl samples were spread over the surface of a blood agar plate containing penicillinase (Penase, 1000 µ/ml). MBC was defined as the lowest concentration of penicillin which reduce the inoculum by >99.9% within 24 hours.

Determination of penicillin tolerance

A mid-logarithmic phase culture of the β-streptococci was inoculated (10µl) in 1.0 ml TY-medium with serial doubling dilutions of phenoxythymethylpenicillin. The zero sample had a bacterial density corresponding 2x10^5 - 5x10^5 cfu/ml. All tubes were incubated at 37°C for 24 hours. The MIC was determined by visual examination for turbidity. Before sampling for MBC the tubes were stirred and 100 µl samples were spread over the surface of a blood agar plate containing penicillinase (Penase, 1000 µ/ml). The plates were then incubated at 37°C for 24 hours. MBC was determined and tolerance was defined as a MBC/MIC ratio >16.
RESULTS AND DISCUSSION

Methodological and epidemiological aspects on bacterial interference (I, II)

Variation in methodology constitutes the basis for evaluation of the significance of different factors involved in bacterial interference, (37) such as pH, accumulation of waste products (like ammonia, lactic acid, free fatty acids), competition for essential growth substances, bacteriophages, peroxide production, bacterial toxins, enzymes and bacteriocins (37). In the present study (I) interference was analyzed on agar plates using the Steers' steel pin replicator (66). The plates were incubated under anaerobic as well as aerobic conditions in order to eliminate the effect of peroxide production (75, 76).

It was found that the α-streptococci incubated under anaerobic conditions had the same inhibition pattern as that under aerobic conditions except for the strain α29 that had no or slight inhibiting capacity under anaerobic conditions. The same factor might also have influenced upon the results obtained in broth, however, this method, also used by Beck (41) had the advantage of allowing quantitation and timing of interference.

A discrepancy between the interference on aerobic agar plates and that registered when using the broth method was also noted. GAS T12 was not inhibited by α1 and α29 in broth, but a good inhibition was noted on agar plates. This might have been influenced by differences in inoculum size or phase of growth of either the α- or β-streptococci. This was suppor-
ted by the results from the "pregrowth experiment" in broth which agreed with those of the agar plates with one exception (α4 together with GAS T12). Also Sanders (38) demonstrated a good correlation between the screening procedure on agar plates with those obtained in broth.

The method with "agar overlay plates" was made to eliminate some of the unspecific factors as pH-changes, lack of nutrients and bacteriophages (9,77,78). The initial pH on the surface of the blood agar plates diminished from 7.5 to 7.1 during the 18 h of incubation of the α-streptococcal strains. After the second agar layer the pH was 7.4-7.5 after gelification and diminished after incubation with a GAS T4 strain similar to the incubation with the α-streptococci. The results of the interference experiments employing this agar overlay technique agreed with the results obtained by interference on agar plates and pregrowth in broth.

It was also possible to verify the in vitro observed interference in the in vivo model (67) with one single exception. In this latter situation α1 did not inhibit the growth of GAS T12 which was observed using the plate or the broth method.

When comparing the three methods it was registered that the inhibiting capacity of an α-streptococcal strains on group A streptococci belonging to the same serologic type varied within a wide range. It seems likely that this almost fingerprint-like interfering capacity should be referred to the presence of bacteroin-like substances. The influence by most of
the other interfering substances could probably be ruled out due to the techniques used although admittedly factors like accumulated biologically active waste products might still have interfered in the interplay between the microorganisms. Preliminary experiments using ultrafiltration indicated a molecular weight of <10,000 (figure 3) corresponding to the molecular size noted in several other purified streptococcal bacteriocins (81,83,84). Other bacteriocin-like substances released in vitro by Streptococcus sanguis (79,80), S.mitis (44), S.salivarius (81) and S.mutans (46) have a higher molecular weight.

In our study of GAS tonsillitis in an epidemic outbreak the frequency of α-streptococcal strains with inhibiting activity on β-hemolytic streptococci was lower in children that got β-streptococcal infections than in those not developing tonsillitis. (II). Furthermore the number of inhibiting α-streptococci was increased after a GAS infection. These results confirm the work of Crowe et al (9) and Sanders et al (39).
Several examples of how the inhibitory effect of the α-streptococcal flora might have positively influenced the protection against group A tonsillitis can be given in our study. Thus, a sister to one of the children with recurrent β-streptococcal tonsillitis had α-streptococcal strains with broad inhibitory capacity on the β-streptococci tested. She never showed any signs of infection and was constantly negative for β-streptococci in throat cultures. Sprunt et al (42) implanted an α-streptococcal strain for protection against pathogenic microorganisms in nasopharynx of 22 infants, and a conversion of the flora to normal was seen. Then, at least under certain circumstances α-streptococci can play a decisive role in the protection against β-streptococcal tonsillitis.

Bacterial interference in treatment failures (III)
Bacterial interference between α- and β-hemolytic streptococci in patients with clinical treatment failures were compared with that of healthy carriers in their families. The patients with clinical treatment failures had significantly less α-streptococci (2/13) with inhibiting activity against their own β-streptococci than the healthy carriers (11/12) harbouring the same β-streptococcal type. β-streptococci with inhibiting activity against their own α-streptococcal flora were more often found in patients with clinical treatment failures than in healthy carriers (table 2). This further indicates that α-streptococci with inhibiting capacity on β-streptococci may be of importance for resistance to β-streptococcal tonsillitis.
Table 2

Inhibiting activity of α- and β-streptococci in patients with clinical treatment failure and their healthy controls (contacts)

<table>
<thead>
<tr>
<th>Patient group and contacts</th>
<th>Number of persons</th>
<th>Frequency of α-streptococci inhibiting their own β-streptococcal strain</th>
<th>Frequency of β-streptococci inhibiting their own α-streptococcal strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical treatment failures</td>
<td>12 (+1*)</td>
<td>2 (17%)</td>
<td>11 (92%)</td>
</tr>
<tr>
<td>Healthy contacts (carriers)</td>
<td>12</td>
<td>11 (92%)</td>
<td>6 (50%)</td>
</tr>
</tbody>
</table>

* One of these patients completely lacked α-streptococci

**Beta-lactamase activity in saliva (III)**

Beta-lactamase activity in the saliva sediment (after centrifugation of mixed saliva) was more often found in the group of clinical treatment failures than in any other group of patients (table I, paper III). Thus, seven out of 11 patients (64%) had beta-lactamase activity in their saliva sediment. Two of these seven also had β-lactamase activity in the saliva supernate. This seems to indicate that beta-lactamase activity in saliva may be of a clinical significance in patients with clinical treatment failures. In recent years, several studies (31,32,34,36,85) have been presented that indicate that betalactamase production by aerobic and anaerobic strains may influence on the result of penicillin therapy. Brook et al (36) investigated the activity of beta-lactamase in tonsillar tissue from children with recurrent tonsillitis. He found beta-lactamase activity in 33 of 39 tonsils that harboured beta-lactamase producing bacteria. No attempt was made in the
present study (III) to correlate the beta-lactamase activity in saliva with beta-lactamase producing bacteria on or in the tonsils. However, in the control study sediments from saliva containing $>10^5$ highly beta-lactamase producing bacteria (B. fragilis, B34) per ml gave a positive reaction while $<10^9$ bacteria per ml of a low producer gave a negative result. The predominant anaerobic beta-lactamase producing organisms in tonsils of children with recurrent tonsillitis are Bacteroides species and Fusobacterium species (31, 32). Heimdahl et al (85) found clinical failures associated with beta-lactamase producing strains of Bacteroides during treatment of orofacial infections with penicillin. Bacteroides melaninogenicus and Bacteroides oralis can also protect Fusobacterium necrophorum from penicillin in vivo due to beta-lactamase production (86). Recently Tunér et al (87, 88) isolated a Fusobacterium nucleatum from a patient with recurrent tonsillitis. This strain was a highly active producer of beta-lactamase, which they were able to isolate and purify. Additional information on beta-lactamase in saliva was recently obtained in an ongoing study by Grahn and Holm, who found that saliva from persons with saliva pellet positive for beta-lactamase inactivated phenoxy-methylpenicillin more than saliva from persons with a beta-lactamase negative pellet.

Penicillin concentration in saliva and serum (IV)

In a cross-over study comprising 20 persons, a single dose of 12.5 mg/kg body weight phenoxy-methylpenicillin was given as regular coated tablets. One week later the same dose was given but as extra coated tablets. The highest concentration in saliva was registered immediately (2 minutes) after swallowing
the regular coated tablets, mean 2.52 mg/l, followed by a rapid drop during the subsequent 30 minutes. A second peak was seen 60 minutes after the administration and reached 0.1 mg/l (mean). These findings differ from those reported by other authors (17,20, 23) since they started the saliva sampling not until 30-60 minutes after the oral intake of the tablet and could therefore not register the initial peak. Persons given extra coated tablets had a significantly lower initial peak, mean 0.55 mg/l, that already after 5 minutes had diminished to a concentration near the MIC for the α-streptococci (0.16 mg/l). The serum concentration curves for the two types of tablets were equivalent with a peak at 60 minutes 3.46 mg/l and 3.52 mg/l) for ordinary and extra coated tablets respectively. A negative long lasting effect on the normal flora by antibiotics and especially phenoxyethylpenicillin has been demonstrated (8, 23, 40). We noted (IV) a decrease in the number of α-streptococci already 240 minutes after administration of phenoxyethylpenicillin, but this effect on the normal flora was more pronounced using ordinary penicillin tablets than that registered using tablets containing an extra layer of glucose intended to protect against an early dissolution of penicillin in the mouth (Table 1, paper IV). It thus seems likely that the initial saliva peak - representing the penicillin dissolved in saliva during the swallowing process - should be closely observed to avoid ecological disturbances in the throat.
Influence of penicillin on the bacterial interference 
in vitro (IV)

The interference between α- and β-hemolytic streptococci were studied in TY-broth in the absence and presence of different concentrations of phenoxyethylpenicillin (0.01-10 mg/l). In absence of penicillin the α1 strain killed the GAS T4 lab strain within 8 hours after the start of the experiment. If the strains were grown separately both bacteria increased to $>10^8$ cfu/ml in 24 h (fig 4A and 4A, paper IV). When the two bacteria were grown at a low penicillin-concentration (0.01 mg/l) the α1 strain grew almost uninhibited while the GAS T4 lab strain was not recovered after 3 hours of incubation (fig 4B). In the higher penicillin concentration (1.0 mg/l) both bacteria species declined significantly during the 24h but could still be isolated at this time. This indicates a more rapid killing in the presence of low concentration of penicillin and inhibiting α-streptococci compared to the finding using higher concentrations of penicillin. This synergistic effect on killing of GAS by low concentration of phenoxyethylpenicillin and inhibition of α-streptococci can probably be explained by the presence of a bacteriocin produced by the α-streptococci, similar to the one reported by Dajani et al (44). A similar result was found with another α-streptococcal strain (a2155) although this α-strain had a less pronounced inhibitory effect on GAS T4clin. However, if the penicillin concentration was increased further (10 mg/l) viable GAS were still present 24 hours later while no α-streptococci could be demonstrated. This survival of β-streptococci in penicillin concentrations corresponding to 500-1000xMIC simulates the paradoxical effect of antibiotics described by Eagle.
and Musselman (89) They suggested that this phenomenon was due to interference with protein synthesis. An alternative explanation to the paradoxical effect has been presented by Tomaz (90) and Sabath (56) who suggested that a defect in the autolytic system was responsible.

In order to imitate the pharmacological kinetics in saliva after intake of phenoxyethyl-penicillin tablets, a2155 together with GAS T4 lab were exposed to a high pc-V concentration, 2.5 mg/l and also 0.5 mg/l for 10 minutes. Then the pc-V level was lowered by dilution with TY-medium from 2.5 to 0.1 mg/l and from 0.5 to 0.03 mg/l respectively (fig. 6, paper IV). The GAS T4 lab strain decreased earlier when grown in the lower penicillin concentration compared to when it was cultivated in the higher concentrations. The α-streptococci was also influenced during a 8 hours period especially after exposure to high pc-concentrations. However, after this time a regrowth was demonstrated. Earlier reports (9 and paper II) have shown, that resistance versus infection with β-streptococci in the throat, correlates well with the presence of an inhibitory α-streptococcal flora. Since the choice of antibiotic concentration used in paper IV was based on antibiotics levels usually reached in saliva and tonsillar tissue (18) it is tempting to suggest that the results presented might be of clinical significance, but the translation of in vitro results to the clinical situation should be done with extreme caution. The post antibiotic effect of penicillin on β-hemolytic streptococci (91), the known effects of subMIC levels of penicillin on growth and attachment (92) as well as the synergistic killing effect on β-streptococci by low concentration of
penicillin and inhibition of α-streptococci indicate that there is an intricate interplay between several factors in the throat during penicillin treatment. It is suggested that high antibiotic concentrations in the throat should be avoided in view of the negative effects demonstrated.

The effect of penicillin on the bacterial interference in vivo (V)
To study the interplay between α- and β-hemolytic streptococci under in vivo conditions during penicillin treatment rabbits with inoperated steel net chambers were used. The rabbits were treated with high or low dose benzylpenicillin i.m. and the pharmacokinetics of penicillin in the tissue cages were followed. The advantages of the tissue cage model are the composition of tcf and the possibility of repeated sampling. The penicillin concentration in tcf from rabbits treated with 0.5 mg/kg benzylpenicillin i.m. (low dose) reached 0.026 mg/l at 120 minutes as the highest value while rabbits given 10 mg/kg reached the peak value 0.25 mg/l at 60 minutes.

In the untreated rabbit a strong interfering capacity of α-streptococci was seen resulting in killing of the GAS T4 lab strain using either of the two α-streptococcal strains (a2155, a1). Also the GAS T4 clin strain was clearly inhibited but not killed. The aim was to reach a concentration above the MIC of the GAS strains (0.02 mg/l) but below that of the inhibitory α-streptococcal strains (0.1-0.2 mg/l) since a synergistic effect between penicillin and inhibiting α-streptococci on the killing rate of β-streptococci was observed in the in vitro study (paper IV). Using 10 mg/kg of benzylpeni-
cillin twice a day the GAS as well as the α-streptococci were killed within 72 hours of therapy. The pc-G concentration reached the MIC for the α-streptococci during 1-2 hours and this short period was obviously enough to kill the α-strep­tococci. Using the lower dose of penicillin (0.5 mg/kg) we reached a concentration below the MIC for the α-streptococci in the tcf, and near the MIC for the β-streptococci. We could then observe a synergistic effect between penicillin and living α-streptococci, illustrated by a faster killing of GAS T4 lab strain. The present results and those earlier reported (paper II, III, IV) indicate that also the presence of high penicillin levels during a short time should be avoided in order to protect the individual against ecological disturbances with profound biological effects (8, 40). Therefore it seems to be little reason to increase the dose as long as the β-streptococci remain fully sensitive to penicillin.

Penicillin tolerance and sensitivity of β-streptococci to other antibiotics (VI)
The possibility for a decreased sensitivity to penicillin among GAS strain should always be considered a cause to therapeutical failures because penicillin tolerant β-streptococci have been described recently (57-62). The MIC of penicillin for all 33 strains isolated from patients with clinical treatment failure and patients sucessfully cured were 0.01-0.02 mg/l. β-streptococci tolerant to penicillin (MBC >16 times the MIC) were registrated in 11 (61%) of the 18 strains from the clinical treatment failure group but in none of the 15 strains from the group of sucessfully treated patients (table 3). The sensitivity vs other antibiotics for the 18 strains
from the treatment failure patients (table 2, paper VI) demonstrated a MIC for clindamycin of 0.03-0.06 mg/1, cefadroxil 0.25-0.5 mg/1, erythromycin 0.03 mg/1 and for doxycyclin 0.5-2 mg/1, but 2 strains were resistant (MIC = 25 mg/1). In other reports from Sweden streptococci resistant not only to tetracycline, but also to erythromycin have been demonstrated (54, 55). The MBC/MIC ratio of these four antibiotics in the treatment failure group was <16 for all strains versus cefadroxil. This ratio was >16 in 4/18 strains versus clindamycin, 9/18 vs doxycyclin and 3/18 vs erythromycin. A similar pattern was seen among the strains from the control group.

Table 3

Tolerance in β-streptococci vs phenoxyethylpenicillin

<table>
<thead>
<tr>
<th>Patient groups</th>
<th>MBC/MIC &gt;16</th>
<th>MBC/MIC &lt;16</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;Clinical treatment failures&quot;</td>
<td>11</td>
<td>7</td>
</tr>
<tr>
<td>&quot;Controls&quot; *</td>
<td>0</td>
<td>15</td>
</tr>
</tbody>
</table>

*Tonsillitis patients cured with phenoxyethylpenicillin

Different methods and definitions have been used to define penicillin tolerance (56,59,93,94). One problem is demonstrated in table 4, paper VI. The β-streptococcal strain illustrated has a MIC of 0.01 mg/1 but the MBC can be read at 0.01 mg/1 (MBC/MIC ratio = 1) or at 10 mg/1 (MBC/MIC ratio >1000)
since at both those concentrations a killing effect of > 99.9% was noted. Because of a decreased killing at higher concentrations this very sensitive strain should be designated as tolerant according to the definition. However, this decreased killing at higher concentrations is probably an effect of the paradoxical phenomenon described by Eagle and Musselman (89). Also some tolerant strains showed a similar paradoxical effect and it should not be excluded that the two phenomenon are related. Tolerance does not seem to be directly related to resistance to other drugs since the same frequency of "tolerance" against other antibiotics was seen among the penicillin tolerant and non tolerant strains. It is not unlikely that the tolerant bacterial has a penetration barrier to the penicillin molecule. However, also a defect in the autolytic system (56, 90) or altered penicillin binding proteins (95) may be alternative explanations for tolerance. In a similar way a change in the affinity for penicillin by various penicillin binding proteins in the cell wall (peptidase enzymes) has been suggested as an explanation to the Eagle phenomenon (96).

There have been some recent reports indicating that penicillin tolerance may be associated with treatment failure (62-64, 97,100) and our study based on patients with acute streptococcal tonsillitis also indicates that tolerance phenomenon might be one of several reasons to treatment failures in tonsillitis patients given penicillin.
GENERAL SUMMARY

In this thesis based on in vitro experiments, an in vivo experimental model and patients with acute streptococcal tonsillitis it was shown that:

- the Steers' steel pin replicator was a useful tool to study the interference between α- and β-hemolytic streptococci on blood agar plates for screening purpose.

- several α-strains having inhibitory capacity to the majority of group A streptococci belonging to different serotypes were found, but also α-strains with an inhibitory capacity restricted to few group A isolates within a certain serotype.

- a good correlation was found between the different methods for interference studies and the results indicate that the main inhibitory capacity of the α-streptococci most likely is attributed to bacteriocin-like substances.

- in an outbreak of streptococcal tonsillitis, strains of α-hemolytic streptococci with a strong inhibitory capacity on β-streptococci were isolated mainly from individuals seemingly resistant to β-streptococcal tonsillitis, while from patients with repeated tonsillitis no or low numbers of inhibiting α-streptococci were demonstrated.
- in patients with clinical tonsillitis, β-streptococci with inhibiting activity on the patients own α-streptococcal flora were isolated.

- the percentage of inhibiting α-streptococci increased after infection with group A streptococci.

- patients with clinical treatment failure had less (2/13) α-streptococci with inhibiting capacity on their own β-streptococcal strain compared with the healthy carriers (11/12). Furthermore, 6 out of 12 healthy carriers had β-streptococci with inhibiting capacity on their own α-streptococcal flora, while the β-streptococci in 11 out of 12 clinical treatment failures had this ability vs their α-streptococci.

- patients with clinical treatment failure showed beta-lactamase activity in their saliva pellet significantly more often than in the control groups.

- penicillin was released from ordinary sugar coated tablets already in the mouth resulting in concentrations in saliva above the MIC of most penicillin sensitive microorganisms in the throat during the first two minutes. This was followed by a decrease of the α-streptococcal flora.

- a synergistic effect on the killing rate of GAS by low concentrations of penicillin and inhibition of α-streptococci could be demonstrated in vitro.
- high pc-V concentration (500-1,000 x MIC) did not always kill the β-streptococci within 24 h (Eagle's paradoxical effect) in vitro.

- in the experimental animal studies, higher penicillin concentration did not increase the killing rate of β-streptococci, while a faster killing was observed with low penicillin levels in the presence of inhibiting β-streptococci, indicating synergistic effect, also in vivo.

- penicillin tolerance was registered in 11/18 β-streptococci from the treatment failure group, but in none of the 15 strains from the group of successfully treated patients.
GENERAL REMARKS AND CONCLUSION

Several factors have been connected to treatment failures in streptococcal tonsillitis like:

- inadequate penicillin concentration in the site of infection due to e.g. bad patient compliance, inadequate dosage, too short duration of therapy, decreased penetration of antibiotic into the tonsillar tissue, inactivation of penicillin by beta-lactamase producing bacteria in and outside the tonsils
- disturbances in the normal throat flora
- penicillin tolerance among β-streptococci
- reinfection by family members or other contacts
- immunological defects.

In this study it was shown that bacterial interference of the normal throat flora on β-hemolytic streptococci plays an important role for the protection against tonsillitis as well as against renewed infections. We also demonstrated that oral treatment with penicillin in generally recommended doses was followed by a decrease of α-streptococci, due to the high penicillin concentration in saliva found immediately after intake of the tablets. Penicillin at these levels induced a negative effect on the interference of α-streptococci on β-streptococci. On the other hand a synergistic effect on the killing rate of β-streptococci by low concentration of penicillin and inhibition of α-streptococci was demonstrated.
High penicillin concentrations may not only decrease the α-streptococcal flora but also affect the selection of beta-lactamase producing strains in the throat. Betalactamase activity in saliva pellet was more often found in patients with clinical treatment failure, indicating that this beta-lactamase activity may protect the β-streptococci from the action of penicillin. The presence of penicillin tolerant β-streptococcal strains in high frequency in treatment failures might also be a consequence of repeated penicillin therapy.

Several bacteriological factors, (bacterial interference, beta-lactamase, penicillin tolerance) are involved in clinical treatment failures of streptococcal tonsillitis using phenoxy-methylpenicillin. It thus seems likely that the high failure rate represent the result of a sequential and simultaneous action of these factors.

Therefore it is reasonable to suggest that:

- high penicillin levels are not always an advantage in the treatment of streptococcal tonsillitis, and might hinder the interference normally expressed by bacteria in the normal flora.

- an alternative to penicillin should be considered in the treatment of patients with clinical treatment failures, due to penicillin tolerance and β-lactamase production.
ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to:

Stig Holm for his active support, invaluable advice, constructive criticism and never failing encouragement in spite of my two pregnancies in the middle of my scientific education,

Kristian Roos for collaboration and friendship,

Karin Näslund, Eva Norrman, Marie Eklund and Britt-Marie Sundberg for excellent technical assistance,

Ingegärd Eklöf, Christine Boström and Anne-Lie Persson for excellent secretarial assistance,

Stig Granström and Åsa Nordqvist for loyal support and practical advise,

Rolf Sjöberg for photography,

Tord Persson and Melker Söderström for taking care of the rabbits in an excellent way,

The staff of the Department of Clinical Bacteriology for their kind support and for all the nice time we have spent together,

The volunteers who swallowed penicillin-tablets and delivered saliva samples,

The families in Gimonäs for their patience with the repeatedly throat samplings,

My mother for her encouragement,

Kenneth, Andreas and Johan.

This study was supported by a grant from AB Leo Research Foundation, Helsingborg, Sweden and from the Medical Faculty, University of Umeå, Sweden.
REFERENCES


