Incidence of penicillin tolerance among blood culture isolates of *Streptococcus sanguis*, 1987-88

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Abstract

Laboratories that reported isolations of *Streptococcus sanguis* from blood cultures to the Communicable Disease Surveillance Centre (CDSC) Colindale were requested to submit strains to Bath Public Health Laboratory to allow the prevalence of penicillin tolerance within different biotypes of this species to be studied. One hundred and fifty one *Streptococcus* spp were received from 78 United Kingdom laboratories in one year. Strains were identified using the API 20 Strep, and minimum inhibitory concentrations (MICs) of penicillin were determined using the spiral gradient plate method. Penicillin tolerance was detected by spraying β -lactamase over inoculated gradient plates, reincubating for 48 hours and counting the number of surviving organisms represented by colonies. There were 57 different API identification profiles encountered in the survey. Most *S sanguis* I/1 strains were penicillin tolerant, most *S sanguis* II strains were non-tolerant. The overall geometric mean MIC of penicillin was considerably lower for *S sanguis* I/1 than for all other biotypes. The distribution of biotypes and the geometric mean MIC of penicillin for each biotype were not significantly different for infective endocarditis strains than for all strains tested, suggesting little or no association between penicillin tolerance and the seeding of endocardium. When the reactions obtained using API 20 Strep were compared with a recent taxonomic study of viridans streptococci, 22 of 38 *S sanguis* I/1 strains could be reclassified as *S gordonii*; all these strains were penicillin tolerant. Such reclassification would allow likely penicillin tolerant strains to be predicted.

Penicillin tolerance was first described by Tomasz in 1970 in a laboratory mutant of *Streptococcus pneumoniae*.¹ It has been defined as a minimum bactericidal:minimum inhibitory concentration ratio of >32 ,² and although this definition has been criticised,³ it has been used extensively to differentiate tolerant from non-tolerant organisms. There are conflicting reports of the incidence of penicillin tolerance in streptococci,⁴ though the irreproducibility of conventional minimum inhibitory concentration (MIC) and

minimum bactericidal (MBC) methods, particularly for streptococci,⁵ due to various technical factors,⁶ probably account for such discrepancies.

The clinical importance of penicillin tolerance is still uncertain, though it is likely to be of more importance in deep-seated infections such as infective endocarditis, where the use of bactericidal antibiotics is essential for a favourable clinical response.⁴ There are numerous case reports of streptococcal infections, particularly infective endocarditis, in which penicillin tolerance has been proposed as a possible explanation for treatment failure or poor clinical response.⁷⁻¹⁷

Streptococcus spp accounted for 4.4% of significant positive blood cultures reported to the Communicable Diseases Surveillance Centre (CDSC) from April 1987 to March 1988. *Streptococcus* spp account for more than 60% of cases of infective endocarditis,¹⁸ of which *S sanguis* is the most frequently isolated species.¹⁹

Because of the importance of *S sanguis* in infective endocarditis, a study was undertaken to establish the incidence of penicillin tolerance in this species. We report several interesting findings relating to certain biotypes of *S sanguis*. Because *S mitis* is taxonomically very close to *S sanguis* II,²⁰ organisms identified as *S mitis* were included in the study.

Methods

Laboratories reporting isolations of *S sanguis* from blood cultures to the CDSC between April 1987 and March 1988 were requested to forward isolates to Bath Public Health Laboratory together with brief clinical details. On receipt, cultures were checked for purity and then stored at -70°C in glycerol broth. On completion of the collection, all organisms were recovered from -70°C and subcultured several times on blood agar plates incubated at 37°C in 7% carbon dioxide. Each isolate was identified using the API 20 Strep (API, bioMérieux) in accordance with the manufacturer's instructions.

The API 20 Strep kits consist of plastic strips each with 20 microtubes containing substrates for detection of biochemical reactions in the following order: acetoin production (VP); hippurate hydrolysis and aesculin hydrolysis; production of pyrrolidonylarylamidase, α -galactoside, β -glucuronidase, β -galactosidase, alkaline phosphatase, leucine arylamidase, and arginine dihydrolase; acidification of ribose, L-arabinose, mannitol, sor-

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Table 1 Blood culture isolations of viridans streptococci (laboratory reports to CDSC April 1987–March 1988)

Organism	Total number	With infective endocarditis number (%)
<i>S. milleri</i>	203	17 (8.7)
Streptococcus group F	15	2 (26.3)
<i>S. mitis</i> (<i>mitior</i>)	194	51 (78.6)
<i>S. mutans</i>	28	21 (32.3)
<i>S. salivarius</i>	65	136 (36.3)
<i>S. sanguis</i>	375	46 (23.5)
α and non-haemolytic streptococci	196	295 (27.4)
Total	1076	

Table 2 Distribution of biotypes of *S. sanguis* from blood culture isolates received for investigation

	All isolates	Presumed infective endocarditis	Suspected infective endocarditis
<i>S. sanguis</i> biotype I	57	17	25
<i>S. sanguis</i> biotype II	67	19	21
All <i>S. sanguis</i>	124	36	46

Table 3 Number of strains tolerant for each API profile obtained

Identification	API profile	Identification %	No of strains	No of tolerant strains
<i>S. sanguis</i> I	0041611	91.8	1	1
	0241441	(*)	1	0
<i>S. sanguis</i> I/?	0041451	81.2	1	0
	4241450	90.8	2	1
<i>S. sanguis</i> I/1	0041410	89.8	1	1
	0041430	98.1	2	1 (1)
	0041630	79.5	1	1
	0061430	97.3	1	1
	0241050	71.7	1	0 (1)
	4040410	87.1	1	1
	4041410	99.6	1	0
	4041430	99.8	3	2
	4041630	87.2	1	1
	4060411	95.0	2	0
	4060430	98.4	1	1
	4061410	96.9	3	3
	4061430	99.6	17	17
	4061431	98.3	1	1
	4071410	98.5	1	1
	4261470	72.1	1	1
<i>S. sanguis</i> I/2	0041631	79.3	1	0 (1)
	0041671	99.0	1	0 (1)
	0241251	98.1	1	0 (1)
	0241471	75.4	1	1
	0241651	99.5	1	1
	4241451	93.7	4	3
	4241470	77.5	1	0
	4241471	94.9	1	1
	4241650	99.3	1	1
	4241670	99.6	1	1
	4241671	99.9	1	1
<i>S. sanguis</i> II	0240440	(*)	1	0
	0240441	97.8	5	0
	0240451	97.8	1	0
	0241441	(*)	1	0
	0241451	(*)	1	0 (1)
	0242451	83.4	1	0
	0251441	(*)	1	0
	0260440	99.6	2	0 (2)
	0260441	99.9	26	0
	0260451	99.3	12	0
	0260460	(*)	1	0
	0260641	98.1	2	0
	0261440	96.8	7	3 (2)
	0261441	98.4	3	1
	0261450	73.7	1	1
	0261461	72.1	1	0 (1)
	0270450	98.6	1	0
<i>S. mitis</i>	0040400	87.1	1	0
	0040401	98.2	3	0
	0040420	89.3	1	0
	0041400	(*)	2	0
	0041401	84.4	3	1
	0041421	(*)	1	1
	0060401	99.1	1	0
	0060410	79.1	2	1
	0060411	91.2	2	1

(*) Identification confirmed by API bioMérieux laboratories. Numbers in parentheses indicate strains that were tolerant only in the stationary phase of growth.

bitol, lactose, trehalose, inulin, raffinose, starch and glycogen. The final test used was the production of haemolysis on blood agar. A seven digit profile number was obtained by assigning each group of three tests a numerical value of 1, 2, and 4 sequentially and scoring positive tests within each group. Profile numbers obtained after 24 hour incubation were translated to organism identifications using APILAB software version 1.3. The identification for organisms that gave "identification percentages" of below 70% was confirmed by API bioMérieux laboratories using API 50CH.

Minimum inhibitory concentrations of penicillin were determined using the Spiral Gradient Endpoint (SGE) method, which we have previously shown to be a reproducible method for MIC determinations with *Streptococcus* spp.⁵ Penicillin gradients covering a range of concentrations from 120 mg/l to 0.001 mg/l over two plates were established using a spiral plater (Spiral Instruments) on 140 mm Petri dishes containing Iso-Sensitest agar (Oxoid) with 1% lysed horse blood to a depth of 3.3 mm. Starting concentrations used to establish the gradients were 19500 mg/l and 47 mg/l.

Overnight BHI (Oxoid) broth cultures (stationary phase) were diluted 10-fold in warm BHI broth and incubated further for one to two hours at 37°C to obtain log-phase cultures. Each set of gradient plates was inoculated with five different clinical isolates in both log and stationary phases of growth from the periphery to within 20 mm of the centre using sterile cotton-tipped swabs. Tolerant (San 1303) and non-tolerant (San 2131) control strains with known MICs to penicillin were included on every plate. The plates were incubated at 37°C in 7% carbon dioxide for 24 hours. After incubation the endpoints of growth were marked on the reverse of the plate with a felt-tipped pen. The plates were then sprayed with 1.0 ml of 50 U/ml broad spectrum β -lactamase (Porton Products) and reincubated for 48 hours after which the plates were examined for regrowth of inhibited cells. Greater than 10 colonies above the MIC endpoints was taken to be indicative of penicillin tolerance.

Results

The number of blood culture isolates and the number of cases of infective endocarditis reported to the CDSC during the study period are shown in table 1. A total of 151 *Streptococcus* strains were received from 78 United Kingdom laboratories during the study, 124 identified as *S. sanguis*, 16 as *S. mitis*, and the remaining 11 were various other species. The distribution of *S. sanguis* biotypes from presumed and suspected cases of infective endocarditis and other bacteraemias is shown in table 2. In this series the distribution of *S. sanguis* biotypes I and II, overall, and from cases of infective endocarditis was roughly equal.

Spiral gradient endpoint plate inoculated with three tolerant *S. sanguis* I strains (top) and three non-tolerant *S. sanguis* II strains (bottom). The plate was incubated at 37°C for 24 hours, the endpoints of growth marked, and the plate sprayed with β -lactamase and reincubated for 48 hours. The tolerant strains show regrowth of colonies beyond the MIC endpoints.

Table 4 Incidence of penicillin tolerance in organisms tested, with geometric mean MICs for tolerant and non-tolerant strains

Organism	No tolerant/ No tested	Geometric mean MIC of penicillin (mg/l)		
		Tolerant	Non-tolerant	All
<i>S. sanguis</i> I/1	32/ 38	0.0066	0.12	0.0073
<i>S. sanguis</i> I/2	9/ 14	0.025	0.020	0.023
<i>S. sanguis</i> II	5/ 67	0.066	0.023	0.026
All <i>S. sanguis</i>	48/124	0.011	0.023	0.018
<i>S. mitis</i>	4/ 16	0.034	0.052	0.047

Fifty seven different API profiles were encountered in the study (table 3). *S. sanguis* I/1 strains with API profiles ranging from 4060430–4241450 (24 strains) all gave tolerant responses to penicillin in the logarithmic phase of growth whereas *S. sanguis* II strains with API profiles ranging from 0242451–0260641 (45 strains) all gave non-tolerant responses. This association of tolerance with biotype I strains and non-tolerance with biotype II strains was highly significant (χ^2 test, $p > 0.001$). The figure shows a gradient plate illustrating the different responses of tolerant *S. sanguis* I/1 strains and non-tolerant *S. sanguis* II strains to the killing action of penicillin.

All strains that were tolerant in the logarithmic phase of growth were also tolerant in the stationary phase of growth; 11 strains were tolerant only in the stationary phase (table 3). There was no significant difference (all within the equivalent of one doubling dilution) between MICs in the two phases of growth.

The geometric mean MIC of penicillin for tolerant and non-tolerant *S. sanguis* biotypes

and the number of tolerant strains in each biotype is shown in table 4. Both control organisms which were included on every plate ($n = 37$) gave consistent results. The tolerant control (*S. sanguis* I/3) had a geometric mean MIC of 0.0056 mg/l with a range (2 SD) of 0.0036–0.0086 mg/l, and the non-tolerant control (*S. sanguis* II) had a geometric mean MIC of 0.0643 mg/l with a range (2 SD) of 0.0283–0.1461 mg/l.

Tolerant strains of *S. sanguis* I/1 (which accounted for the majority of tolerant strains) had significantly lower MICs to penicillin than did any other biotype. Conversely, the six non-tolerant strains of *S. sanguis* I/1 had significantly higher MICs than any other biotype. There was no significant difference (all within the equivalent of a doubling dilution) between the overall pattern of geometric MICs (table 4) and geometric MICs of strains from infective endocarditis.

Discussion

The prevalence of different *Streptococcus* spp involved in infective endocarditis was similar to that reported in other British and American studies.¹⁹ It has been suggested that biotype II strains may have greater avidity for endocardium.²¹ We could not show a significant difference in the distribution of biotype II strains from cases of infective endocarditis when compared with the overall distribution (χ^2 test, $p > 0.05$). This observation also indicates that penicillin tolerance is not a factor in establishing the initial infection.

The clustering of tolerant and non-tolerant strains to ranges of API identification profiles for *S. sanguis* I and *S. sanguis* II, respectively, was particularly interesting and suggests that penicillin tolerance is a trait closely associated with certain biotypes and not easily (if at all) transferable to other strains of *Streptococcus* spp. The mode of peptidoglycan cross-linking in these biotypes is believed to be different,²² thus the outcome of penicillin exposure of these biotypes may be a consequence of their different peptidoglycan structures. The difference in cell wall structure may also account for the different geometric mean MICs of penicillin between *S. sanguis* I/1 and *S. sanguis* II strains. It is perhaps not surprising that it has recently been suggested that *S. sanguis* I/1 should constitute a separate species on the basis of DNA homology studies.²⁰ If the reactions obtained with API 20 Strep are compared with those in a recent taxonomic study of viridans streptococci by Kilian *et al*,²³ then most strains identified as *S. sanguis* II would become *S. mitis*, and 22 of the 38 strains of *S. sanguis* I/1 (profiles 4061410–4071410 in table 1) would probably be included in a proposed new species, *S. gordonii*. What is important is that all the strains that we tested which were consistent with the description of *S. gordonii* were penicillin tolerant. It is presently not known whether a patient's response to infection is influenced by the biotype of the infecting strain, though studies using the rabbit model of infective endocarditis provide evidence that

penicillin tolerance is an important factor in the response to antimicrobial chemotherapy.²⁴⁻²⁷ The species and biovar of the infecting strain may therefore have important implications for the course of the disease in man. These implications may become more apparent as the classification of the viridans streptococci is improved.

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- 1 Tomasz A, Albino A, Zanati E. Multiple antibiotic resistance in a bacterium with suppressed autolytic system. *Nature* 1970;227:138-40.
- 2 Sabath LD, Laverdiere M, Wheeler N, Blazevic D, Wilkinson BJ. A new type of penicillin resistance of *Staphylococcus aureus*. *Lancet* 1977;i:443-7.
- 3 Meylan PR, Francioli P, Glauser P. Discrepancies between MIC and actual killing of viridans group streptococci by cell-wall-active antibiotics. *Antimicrob Agents Chemother* 1986;29:418-23.
- 4 Handwerker S, Tomasz A. Antibiotic tolerance among clinical isolates of bacteria. *Rev Infect Dis* 1985;7:368-86.
- 5 James PA. Comparison of four methods for the determination of MIC and MBC of penicillin for viridans streptococci and the implications for penicillin tolerance. *J Antimicrob Chemother* 1990;25:209-16.
- 6 Sherris JC. Problems in in-vitro determination of antibiotic tolerance in clinical isolates. *Antimicrob Agents Chemother* 1986;30:633-7.
- 7 Allen JL, Sprunt K. Discrepancy between minimum inhibitory and minimum bactericidal concentrations of penicillin for group A and group B β -hemolytic streptococci. *J Pediatr* 1978;93:69-71.
- 8 Broughton DD, Mitchell WG, Grossman M, Hadley WK, Cohen MS. Recurrence of group B streptococcal infection. *J Pediatr* 1976;89:183-5.
- 9 Dagan R, Ferne M. Association of penicillin-tolerant streptococci with epidemics of streptococcal pharyngitis in closed communities. *Eur J Clin Microbiol Infect Dis* 1989; 8:629-31.
- 10 Fung JC, Gadbar JJ, Donta ST, Tilton RC. Infective endocarditis due to penicillin-tolerant *Streptococcus bovis*. *Diagn Microbiol Infect Dis* 1986;5:171-6.
- 11 Grahm E, Holm SE, Roos K. Penicillin tolerance in beta-streptococci isolated from patients with tonsillitis. *Scand J Infect Dis* 1987;19:421-6.
- 12 Griffiths LR, Green HT. Paradoxical effect of penicillin in vivo. *J Antimicrob Chemother* 1985;15:507-8.
- 13 Portnoy D, Prentis J, Richards GK. Penicillin tolerance of human isolates of group C streptococci. *Antimicrob Agents Chemother* 1981;20:235-8.
- 14 Savitch CB, Barry AL, Hoepfich PD. Infective endocarditis caused by *Streptococcus bovis* resistant to the lethal effect of penicillin G. *Arch Intern Med* 1978;138:931-4.
- 15 Schauf V, Deveikis A, Riff L, Serota A. Antibiotic-killing kinetics of group B streptococci. *J Pediatr* 1976;89:194-8.
- 16 Smith TD, Huskins CW, Kim KS, Kaplan EL. Efficacy of β -lactamase-resistant penicillin and influence of penicillin tolerance in eradicating streptococci from the pharynx after failure of penicillin therapy for group A streptococcal pharyngitis. *J Pediatr* 1987;110:777-82.
- 17 Streinbreder P. Serious infection in an adult due to penicillin-tolerant group B *Streptococcus*. *Arch Intern Med* 1981;141:1714-5.
- 18 Baylis R, Clarke C, Oakley CM, Somerville W, Whitfield AGW, Young SEJ. The microbiology and pathogenesis of infective endocarditis. *Br Heart J* 1983;50:513-9.
- 19 Scheld MW. Therapy of streptococcal endocarditis: correlation of animal and clinical studies. *J Antimicrob Chemother* 1987;20A:71-85.
- 20 Coykendall AL. Classification and identification of the viridans streptococci. *Clin Microbiol Rev* 1989;2:315-28.
- 21 Coykendall AL, Efstratiou A, Colman G. *Streptococcus sanguis* genotypes in endocarditis. Abstracts of the Annual Meeting of the American Society for Microbiology. Washington DC: ASM, 1986:B37, 30.
- 22 Schleifer KH, Kandler O. Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bact Rev* 1972;36:407-77.
- 23 Kilian M, Mikkelsen L, Henrichsen J. Taxonomic study of viridans streptococci: Descriptions of *Streptococcus gordonii* sp. nov. and amended descriptions of *Streptococcus sanguis* (White and Niven 1946), *Streptococcus oralis* (Bridge and Sneath 1982), and *Streptococcus mitis* (Andrews and Horder 1906). *Int J Syst Bacteriol* 1989; 39:471-84.
- 24 Pulliman L, Inokuchi S, Hadley KW, Mills J. Penicillin tolerance of viridans streptococci delays sterilization of vegetations in experimental endocarditis. *Clin Res* 1980; 28:45A.
- 25 Brennan RO, Durack DT. Therapeutic significance of penicillin tolerance in experimental streptococcal endocarditis. *Antimicrob Agents Chemother* 1983;23: 273-7.
- 26 Hess J, Dankert J, Durack D. Significance of penicillin tolerance in vivo: Prevention of experimental *Streptococcus sanguis* endocarditis. *J Antimicrob Chemother* 1983;11:555-64.
- 27 Kim KS, Bayer AS. Significance of in-vitro penicillin tolerance in experimental enterococcal endocarditis. *J Antimicrob Chemother* 1987;19:475-85.