

1 Table S1. Clinical manifestations of the 522 NHBS invasive infections.

<i>Streptococcus</i> group	Bacteraemia without focus	Intra-abdominal infections	Endocarditis	Pulmonary infections	Bone and joint infections	Skin and soft tissue infections	Urinary tract infections	Upper respiratory tract infections	Central nervous system infections	Others ^a	Total, n (%)
<i>anginosus</i>	58	43	3	26	13	14	5	6	2	2	172 (33.0%)
<i>S. anginosus</i>	33	30	3	10	6	8	4	1	0	2	97 (18.6%)
<i>S. constellatus</i>	22	12	0	7	5	3	1	2	1	0	53 (10.2%)
<i>S. intermedius</i>	3	1	0	9	2	3		3	1	0	22 (4.2%)
<i>bovis/equinus</i>	43	11	12	1	3	2	2	0	2	1	77 (14.8%)
<i>S. gallolyticus</i>	16	3	8	0	1	0	1	0	1	0	30 (5.7%)
<i>S. infantarius</i>	7	1	1	0	0	1	0	0	0	0	10 (1.9%)
<i>S. lutetiensis</i>	7	4	1	0	1	0	0	0	0	0	13 (2.5%)
<i>S. pasteurianus</i>	13	3	2	1	1	1	1	0	1	1	24 (4.6%)
<i>mitis</i>	78	16	20	15	7	3	3	4	0	2	148 (28.4%)
<i>S. australis</i>	2	2	0	0	0	0	0	0	0	0	4 (0.8%)
<i>S. cristatus</i>	2	3	1	0	1	0	0	0	0	0	7 (1.3%)
<i>S. infantis</i>	1	1	0	0	0	0	0	1	0	0	3 (0.6%)
<i>S. massiliensis</i>	1	0	0	1	0	0	0	0	0	1	3 (0.6%)
<i>S. mitis</i>	26	2	4	3	0	1	1	1	0	0	38 (7.3%)
<i>S. oralis</i>	45	6	14	6	6	1	2	2	0	1	83 (15.9%)
<i>S. peroris</i>	1	1	0	2	0	0	0	0	0	0	4 (0.8%)
<i>S. pseudopneumoniae</i>	0	0	1	3	0	1	0	0	0	0	5 (1.0%)
<i>S. sinensis</i>	0	1	0	0	0	0	0	0	0	0	1 (0.2%)
<i>mutans</i>	3	0	2	0	0	0	0	0	0	0	5 (1.0%)
<i>S. mutans</i>	3	0	2	0	0	0	0	0	0	0	5 (1.0%)
<i>salivarius</i>	21	10	2	3	0	1	0	0	2	0	39 (7.5%)
<i>S. salivarius</i>	16	7	2	2	0	1	0	0	2	0	30 (5.7%)
<i>S. thermophilus</i>	1	0	0	0	0	0	0	0	0	0	1 (0.2%)
<i>S. vestibularis</i>	4	3	0	1	0	0	0	0	0	0	8 (1.5%)
<i>sanguinis</i>	38	15	19	6	3	0	0	0	0	0	81 (15.5%)
<i>S. gordonii</i>	10	1	3	0	2	0	0	0	0	0	16 (3.1%)
<i>S. parasanguinis</i>	11	12	2	4	0	0	0	0	0	0	29 (5.6%)
<i>S. sanguinis</i>	17	2	14	2	1	0	0	0	0	0	36 (6.9%)
Total, n (%)	241 (46.2%)	95 (18.2%)	58 (11.1%)	51 (9.8%)	26 (5.0%)	20 (3.8%)	10 (1.9%)	10 (1.9%)	6 (1.1%)	5 (1.0%)	522 (100%)

2 ^a including vascular and catheter-related bloodstream infections (n = 3), endophthalmitis (n = 1) and upper genital infection (n = 1).

3 **Table S2. Comparison of MIC values obtained by BMD and gradient Etest on 505 NBHS.**

Agreement	Benzylpenicillin	Amoxicillin	Cefotaxime	Ceftriaxone
Categorical agreement	97.8%	95.0%	97.8%	94.9%
Minor errors	2.2%	5.0%	NA	NA
Major errors	0%	0%	NA	NA
Very major errors	0%	0%	2.2%	5.1%
Essential agreement	95.1%	94.1%	92.9%	82.9%
Absolute agreement	54.4%	53.5%	55.0%	22.9%
Bias	1.1%	2.9%	24.3%	68.6%

4 NA: not applicable.

5 Interpretation according to EUCAST guidelines v 13.0.

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7 **Table S3. Beta-lactam resistance phenotypes of the 522 NBHS.**

Beta-lactam resistance phenotype, n (%)	<i>Streptococcus</i> group					
	<i>anginosus</i> n=172	<i>bovis/equinus</i> n=77	<i>mitis</i> n=148	<i>mutans</i> n=5	<i>salivarius</i> n=39	<i>sanguinis</i> n=81
Susceptible	172 (100%)	77 (100%)	102 (68.9%)	5 (100%)	28 (71.8%)	39 (48.1%)
Benzylpenicillin	0	0	1 (0.7)	0	2 (5.1%)	0
Benzylpenicillin + aminopenicillins	0	0	8 (5.4%)	0	4 (10.3%)	17 (43.6%)
Benzylpenicillin + aminopenicillins + 3 rd generation cephalosporins	0	0	32 (21.6%)	0	4 (10.3%)	15 (18.5%)
Benzylpenicillin + 3 rd generation cephalosporins	0	0	1 (0.7%)	0	0	0
Aminopenicillins	0	0	0	0	0	9 (11.1%)
3 rd generation cephalosporin	0	0	4 (2.7%)	0	1 (2.6%)	1 (1.2%)

8 Values indicated the number (%) of isolates displaying intermediate susceptibility or resistance to benzylpenicillin and aminopenicillins
9 (amoxicillin or ampicillin), and resistance to 3rd generation cephalosporins (cefotaxime or ceftriaxone) according to EUCAST v 13.0 breakpoints.

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14 **Table S4. Performances of the 1-unit PEN-disk screening for the detection of decreased-susceptibility to beta-lactams.**

<i>Streptococcus</i> group (n)	Sensitivity		Specificity		Positive predictive value		Negative predictive value	
	18 mm	21 mm	18 mm	21 mm	18 mm	21 mm	18 mm	21 mm
<i>mitis</i> (148)	69.6 %	87.0 %	100 %	92.2 %	100 %	83.3 %	87.9 %	94.0 %
<i>salivarius</i> (39)	72.7 %	81.8 %	100 %	75.0 %	100 %	56.3 %	90.3 %	91.3 %
<i>sanguinis</i> (81)	45.2 %	69.0 %	100 %	97.4 %	100 %	96.7 %	62.9 %	74.5 %
Total isolates (n=522)	59.6 %	78.8 %	100 %	95.0 %	100 %	78.8 %	91.0 %	95.0 %

15 ^a Susceptible breakpoints from EUCAST v 12.0 (18 mm) and EUCAST v 13.0 (21 mm).

16 PEN, benzylpenicillin.

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19 **Table S5. Beta-lactam resistance phenotypes of the 40 NBHS isolates falsely categorised as susceptible by the 1-unit PEN disk screening**
 20 **(susceptible breakpoint 18 mm, EUCAST v12.0).**

Phenotype ^a				Isolates n (%)	<i>Streptococcus</i> group		
Benzympenicillin	Aminopenicillins (ampicillin or amoxicillin)	Third-generation cephalosporins (cefotaxime or ceftriaxone)	<i>mitis</i> 14 (35%)		<i>salivarius</i> 3 (7%)	<i>sanguinis</i> 23 (58%)	
I	S	S	2 (5 %)	0	2	0	
I	I	S	14 (35 %)	3	0	11	
I	I	R	8 (20 %)	6	0	2	
I	S	R	1 (2 %)	1	0	0	
S	S	R	6 (15 %)	4	1	1	
S	I	S	9 (23 %)	0	0	9	

21 ^a MIC values determined by broth microdilution.

22 NBHS, non-beta-haemolytic streptococci; S, susceptible; I, susceptible increased exposure; R, resistant.

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24 **Table S6. Beta-lactam resistance phenotypes of the 21 NBHS isolates falsely categorised as susceptible by the 1-unit PEN disk screening**
 25 **(susceptible breakpoint 21 mm, EUCAST v13.0).**

Phenotype ^a				Isolates n (%)	<i>Streptococcus</i> group		
Benzympenicillin	Aminopenicillins (ampicillin or amoxicillin)	Third-generation cephalosporins (cefotaxime or ceftriaxone)			<i>mitis</i> 6 (28.6%)	<i>salivarius</i> 2 (9.5%)	<i>sanguinis</i> 13 (61.9%)
I	S	S	2 (9.5%)	0	2	0	
I	I	S	4 (19.0%)	1	0	3	
I	I	R	2 (9.5%)	1	0	1	
I	S	R	1 (4.8%)	1	0	0	
S	S	R	4 (19.0%)	3	0	1	
S	I	S	8 (38.1%)	0	0	8	

26 ^a MIC values determined by broth microdilution.

27 NBHS, non-beta-haemolytic streptococci; S, susceptible; I, susceptible increased exposure; R, resistant.

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30 **Table S7. Clinical characteristics of the 79 NBHS invasive infections in 2022.**

<i>Streptococcus</i> group	<i>anginosus</i>	<i>bovis/equinus</i>	<i>mitis</i>	<i>salivarius</i>	<i>sanguinis</i>	Total
Sex ratio M/F	1.6	0.9	1.6	(4 males)	2.5	1.5
Age, median (min – max)	50 y (0 d - 97 y)	77 y (0 d - 100 y)	57 y (11 - 95 y)	69 y (65 - 87 y)	85 y (11 - 98 y)	65 y (0 d - 100 y)
Age, interquartile range, in years	31 - 69	62 - 86	40 - 70	ND	49 - 93	43 - 81
Total, n (%)	26 (33%)	21 (27%)	21 (27%)	4 (5%)	7 (8%)	79 (100%)
Adult cases (≥ 18 y), n (%)^a	24 (92%)	19 (90%)	20 (95%)	4	6	73 (92%)
Bacteremia without focus	2 (8%)	12 (57%)	5 (24%)	3	1	23 (29%)
Intra-abdominal infections	5 (19%)	0	5 (24%)	0	0	10 (13%)
Endocarditis	1 (4%)	4 (19%)	2 (10%)	0	1	8 (10%)
Pulmonary infections	4 (15%)	0	2 (10%)	0	2	8 (10% ^o)
Bone and joint infections	2 (8%)	3 (14%)	2 (10%)	1	1	9 (11%)
Skin and soft tissue infections	1 (4%)	0	2 (10%)	0	0	3 (4%)
Upper respiratory tract infections	4 (15%)	0	2 (10%)	0	0	6 (8%)
Central nervous system infections	1 (4%)	0	0	0	0	1 (1%)
Others ^b	4 (15%)	0	0	0	1	5 (6%)
Neonates and infants (birth - ≤ 1 y), n (%)^a	1 (4%)	2 (10%)	0	0	0	3 (4%)
Early Onset Sepsis (0-3 d)	1 (4%)	2 (10%)	0	0	0	3 (4%)
Pediatric cases (1 - 18 y), n (%)^a	1 (4%)	0	1 (5%)	0	1	3 (4%)
Bacteremia without focus	0	0	1 (5%)	0	0	1 (1%)
Others ^c	1 (4%)	0	0	0	1	2 (3%)

31 ^a Percentages are relative to the total isolates within each *Streptococcus* group and were not calculated for the *S. salivarius* and *S. sanguinis*
32 groups because of the small number of cases.

33 ^b Including upper -genital infections (n = 4) and urinary tract infections (n = 1).

34 ^c Including skin and soft tissue infection (n = 1), upper respiratory tract infections (n = 1).

35 d, days; m, months; y, years; ND, not determined.

36 **Table S8. Beta-lactam agents - Susceptibility and MIC distributions of 79 invasive NBHS isolates in 2022.**

<i>Streptococcus</i> group (n; %)	Antimicrobial agent	MIC ^a									MIC interpretation ^b , n (%)		
		≤0.016	0.032	0.064	0.125	0.25	0.5	1	2	≥4	S	I	R
<i>anginosus</i> (26; 33%)	Benzylpenicillin	0	7	10	7	2	0	0	0	0	26 (100%)	0	0
	Amoxicillin	2	2	3	7	10	2	0	0	0	26 (100%)	0	0
	Cefotaxime	1	0	4	4	16	1	0	0	0	26 (100%)	NA	0
	Ceftriaxone	0	0	3	8	14	1	0	0	0	26 (100%)	NA	0
<i>bovis/equinus</i> (21; 27%)	Benzylpenicillin	0	0	8	9	4	0	0	0	0	21 (100%)	0	0
	Amoxicillin	0	1	2	11	7	0	0	0	0	21 (100%)	0	0
	Cefotaxime	0	0	3	8	10	0	0	0	0	21 (100%)	NA	0
	Ceftriaxone	1	2	0	15	3	0	0	0	0	21 (100%)	NA	0
<i>mitis</i> (21; 27%)	Benzylpenicillin	0	1	1	3	2	0	4	0	<u>10</u>	7 (33%)	4 (19%)	10 (48%)
	Amoxicillin	0	1	1	2	1	3	2	1	<u>10</u>	8 (38%)	3 (14%)	10 (48%)
	Cefotaxime	0	0	1	2	3	2	<u>1</u>	<u>1</u>	<u>11</u>	8 (38%)	NA	13 (62%)
	Ceftriaxone	0	1	1	2	3	2	0	<u>3</u>	<u>9</u>	9 (43%)	NA	12 (57%)
<i>salivarius</i> (4; 5%)	Benzylpenicillin	0	0	1	1	1	1	0	0	0	3 (75%)	1 (25%)	0
	Amoxicillin	0	0	1	1	0	0	2	0	0	2 (50%)	2 (50%)	0
	Cefotaxime	1	0	1	0	1	0	<u>1</u>	0	0	3 (75%)	NA	1 (25%)
	Ceftriaxone	0	1	1	0	1	1	0	0	0	4 (100%)	NA	0
<i>sanguinis</i> (7; 8%)	Benzylpenicillin	2	0	2	1	0	0	1	0	<u>1</u>	5 (71%)	1 (14.5%)	1 (14.5%)
	Amoxicillin	0	1	0	2	2	0	0	1	<u>1</u>	5 (71%)	1 (14.5%)	1 (14.5%)
	Cefotaxime	1	1	3	0	0	1	0	0	<u>1</u>	6 (86%)	NA	1 (14%)
	Ceftriaxone	0	2	3	0	1	0	0	0	<u>1</u>	6 (86%)	NA	1 (14%)

37 ^a MICs determined by gradient Etest.

38 ^b MICs interpretation according to EUCAST v 13.0. Susceptible isolates in shaded area, resistant isolates underlined.

39 NA, not applicable; S, susceptible; I, susceptible increased exposure; R, resistant.

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42 **Table S9. Macrolides, lincosamides and streptogramins (MLS) – Susceptibility, resistance phenotypes and genotypes of the 79 invasive**
 43 **NBHS isolates in 2022.**

<i>Streptococcus</i> group	Resistant isolates, n (%)	Disk diffusion phenotype ^{a,b} , n (%)				Resistance determinants, n (%) ^b					
		L/LS _A	M	iMLS _B	cMLS _B	<i>mef</i>	<i>erm</i> (A)	<i>erm</i> (B)	<i>erm</i> (T)	<i>lsa</i> (A)	<i>lsa</i> (B)
<i>anginosus</i> (n=26)	12 (46%)	2 (17%)	0 (0%)	2 (17%)	8 (67%)	0 (0%)	4 (33%)	5 (42%)	1 (8%)	3 (25%)	2 (17%)
<i>bovis/equinus</i> (n=21)	13 (62%)	3 (23%)	0 (0%)	0 (0%)	10 (77%)	0 (0%)	2 (15%)	7 (54%)	1 (8%)	0 (0%)	1 (8%)
<i>mitis</i> (n=21)	13 (62%)	0 (0%)	10 (77%)	0 (0%)	3 (23%)	12 (92%)	1 (8%)	2 (15%)	0 (0%)	0 (0%)	0 (0%)
<i>salivarius</i> (n=4)	2 (50%)	0 (0%)	1 (50%)	0 (0%)	1 (50%)	1 (50%)	1 (50%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
<i>sanguinis</i> (n=7)	5 (71%)	1 (20%)	3 (60%)	0 (0%)	1 (20%)	3 (60%)	1 (20%)	0 (0%)	0 (0%)	1 (20%)	1 (20%)
Total (n=79)	45 (57%)	6 (13%)	14 (31%)	2 (4%)	23 (51%)	16 (36%)	9 (20%)	14 (31%)	2 (4%)	4 (9%)	4 (9%)

44 ^a DD phenotypes were interpreted according to the guidelines on Antibiotic Susceptibility Testing of the French Society for Microbiology (CA-
 45 SFM).

46 ^b Percentages are relative to total resistant isolates within each group.

47 L, resistance to lincosamides; LS_A, resistance to lincosamides and streptogramin A; M, efflux-mediated resistance to C14 and C15 macrolides;
 48 iMLS_B, inducible resistance to macrolides, lincosamides and streptogramin B; cMLS_B, constitutive resistance to macrolides, lincosamides and
 49 streptogramin B; NA: not applicable.

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Table S10. Tetracyclines - Susceptibility and resistance determinants of the 79 invasive NBHS isolates in 2022^a.

<i>Streptococcus</i> group	Resistant isolates, n (%)	Resistance determinant, n (%) ^b		
		<i>tet</i> (M)	<i>tet</i> (L)	<i>tet</i> (O)
<i>anginosus</i> , n=26	10 (38%)	7 (70%)	1 (10%)	4 (40%)
<i>bovis/equinus</i> , n=21	17 (81%)	13 (76%)	8 (47%)	4 (24%)
<i>mitis</i> , n=21	5 (24%)	5 (100%)	0 (0%)	0 (0%)
<i>salivarius</i> , n=4	0 (0%)	-	-	-
<i>sanguinis</i> , n=7	0 (0%)	-	-	-
Total, n=79	32 (41%)	25 (78%)	9 (28%)	8 (25%)

54 ^a Interpreted according to the guidelines on Antibiotic Susceptibility Testing of the French Society for Microbiology.

55 ^b Percentages are relative to total resistant isolates within each group.

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