

Table S1: Characteristics of NH/NP isolates collected by the CNR-Strep between 2007 and 2013.

Isolate*	Patient	Invasive / non-invasive	EOD / LOD ^a	Source ^{b,c}	Syndrome ^d	CSP	ST ^d
20130013	neonate	Invasive	EOD	Blood	Bacteremia	Ib	ND
209800983	neonate	Invasive	EOD	Blood	Bacteremia	III	ND
20131298	neonate	Invasive	EOD	Blood	Bacteremia	VI	ND
CCH1330*	neonate	Invasive	EOD	Blood	Bacteremia	III	17
CCH1352*	neonate	Invasive	EOD	CSF ^b	Meningitis	III	17
CCH1322*	neonate	Invasive	LOD	Blood	Bacteremia	Ia	23
CCH1319*	neonate	Invasive	LOD	Blood	Bacteremia	III	17
CCH1320*	neonate	Invasive	LOD	Blood	Bacteremia	III	17
CCH1336*	neonate	Invasive	LOD	Blood	Bacteremia	III	17
CCH1337*	neonate	Invasive	LOD	Blood	Bacteremia	III	27
20130207	neonate	Invasive	LOD	Blood	Bacteremia	III	ND
CCH1340*	neonate	Invasive	LOD	Blood	Bacteremia	IV	459
CCH1318*	neonate	Invasive	LOD	CSF ^b	Meningitis	III	17
CCH1341*	neonate	Invasive	LOD	CSF ^b	Meningitis	III	17
CCH1333*	neonate	Invasive	LOD	CSF ^b	Meningitis	III	17
206800210	neonate	Invasive	LOD	Blood	Bacteremia	III	ND
20130673	neonate	Non-invasive	-	Gastric fluid	Colonization	IV	ND
211800629	neonate	Non-invasive	-	Gastric fluid	Colonization	III	ND
210800492	neonate	Non-invasive	-	Gastric fluid	Colonization	Ia	ND
210800493	neonate	Non-invasive	-	Gastric fluid	Colonization	Ia	ND
20130069	neonate	Non-invasive	-	Stools	Colonization	Ia	ND
CCH1344*	adult	Invasive	-	Blood	Bacteremia	Ib	8
20130718	adult	Invasive	-	Blood	Bacteremia	Ib	ND
20130856	adult	Invasive	-	Blood	Bacteremia	Ib	ND
CCH1351*	adult	Invasive	-	Blood	Bacteremia	II	2
CCH1345*	adult	Invasive	-	Blood	Bacteremia	II	10
CCH1329*	adult	Invasive	-	Blood	Bacteremia	II	12
CCH1343*	adult	Invasive	-	Blood	Bacteremia	II	22
20121061	adult	Invasive	-	Blood	Bacteremia	II	ND
20130897	adult	Invasive	-	Blood	Bacteremia	II	ND
CCH1347*	adult	Invasive	-	Blood	Bacteremia	III	17
CCH1323*	adult	Invasive	-	Blood	Bacteremia	III	27
209800984	adult	Invasive	-	Blood	Bacteremia	III	ND
20121067	adult	Invasive	-	Blood	Bacteremia	III	ND
20121576	adult	Invasive	-	Blood	Bacteremia	III	ND
CCH1348*	adult	Invasive	-	Blood	Bacteremia	IV	459
20131002	adult	Invasive	-	Blood	Bacteremia	IV	ND
CCH1350*	adult	Invasive	-	Blood	Bacteremia	V	1
CCH1328*	adult	Invasive	-	Blood	Bacteremia	V	1
209800638	adult	Invasive	-	Blood	Bacteremia	V	ND
20121276	adult	Invasive	-	Blood	Bacteremia	V	ND
20121410	adult	Invasive	-	Blood	Bacteremia	V	ND
20121597	adult	Invasive	-	Blood	Bacteremia	V	ND

CCH1334*	adult	Invasive	-	Blood	Erysypelas	III	17
CCH1325*	adult	Invasive	-	Blood	Endocarditis	III	1
CCH1324*	adult	Invasive	-	Blood	Endocarditis	V	1
20121165	adult	Invasive	-	Blood	Bone/joint infection	III	ND
207801032	adult	Invasive	-	Bone biopsy	Bone/joint infection	II	ND
CCH1349*	adult	Invasive	-	Bone biopsy	Bone/joint infection	Ib	1
20131427	adult	Invasive	-	Bone biopsy	Bone/joint infection	II	ND
CCH1331*	adult	Invasive	-	Joint fluid	Bone/joint infection	V	1
CCH1338*	adult	Invasive	-	CSF ^b	Meningitis	III	366
CCH1342*	adult	Non-invasive	-	Sperm	Colonization	III	27
20130156	adult	Non-invasive	-	Urine	UTI ^c	III	ND
CCH1327*	adult	Non-invasive	-	Abscess	Abces	II	28
CCH1326*	adult	Non-invasive	-	Skin	Ulcer	III	17
CCH1339*	adult	Non-invasive	-	Vagina	Colonization	Ia	136
CCH1321*	adult	Non-invasive	-	Vagina	Colonization	III	106
CCH1084*	adult	Non-invasive	-	Vagina	Colonization	III	1
20130765	adult	Non-invasive	-	Vagina	Colonization	III	ND
20131184	adult	Non-invasive	-	Vagina	Colonization	III	ND
CCH1346*	adult	Non-invasive	-	Vagina	Colonization	IV	1
20130064	adult	Non-invasive	-	Vagina	Colonization	IV	ND

*: Sequenced isolates

^a: EOD/LOD: Early Onset Disease; Late Onset Disease

^b: Cerebrospinal Fluid

^c: Urinary Track Infection

^d: ND Not Determined

Table S2: Bacterial strains used in this study.

Strain	Relevant properties	Source
<i>E. coli</i>		
TG1	F', traD36, lacIq, proA+B+, lacZΔ, supE, hsdΔ5	
HB101 / pRK24	Strains for <i>E. coli</i> to GBS conjugation	[1]
NEM3535	HB101 / prK24 + pTCV- <i>erm</i>	This study
NEM3536	HB101 / prK24 + pTCVΩP _{cyl+} - <i>abx1</i>	This study
<i>S. agalactiae</i>		
NEM316	Clinical isolate, Serotype III ST-23	[2]
BM110	Clinical isolate, Serotype III ST-17	[3, 4]
A909	Clinical isolate, Serotype Ia	[5]
2603V/R	Clinical isolate, Serotype V	[6]
515	Clinical isolate, Serotype Ia	[5]
H36B	Clinical isolate, Serotype Ib	[5]
CCH1627	NEM316Δ <i>cylX</i>	This study
CCH1628	NEM316Δ <i>cylD</i>	This study
CCH1605	NEM316Δ <i>cylG</i>	This study
NEM4190	NEM316Δ <i>cylAB</i>	This study
NEM4192	NEM316Δ <i>cylI</i>	This study
CCH10	NEM316Δ <i>cylE</i>	[7]
CCH1675	NEM316Δ <i>cylJ</i>	[7]
CCH1676	NEM316Δ <i>cylK</i>	[7]
CCH1353	CCH1084ΔIS:: <i>cyl</i>	This study
CCH1620	NEM316+IS:: <i>cyl</i>	This study
NEM3566	CCH1328+pTCV- <i>erm</i>	This study
NEM3567	CCH1328+pTCVΩP _{cyl+} - <i>abx1</i>	This study
NEM3568	CCH1331+pTCV- <i>erm</i>	This study
NEM3569	CCH1331+pTCVΩP _{cyl+} - <i>abx1</i>	This study
NEM3570	CCH1350+pTCV- <i>erm</i>	This study
NEM3571	CCH1350+pTCVΩP _{cyl+} - <i>abx1</i>	This study
NEM3564	CCH1324+pTCV- <i>erm</i>	This study
NEM3565	CCH1324+pTCVΩP _{cyl+} - <i>abx1</i>	This study
NEM3244	2603V/R+pTCV- <i>erm</i>	[8]
NEM3249	2603V/R+pTCVΩP _{cyl+} - <i>abx1</i>	[8]

Table S3: Plasmids used in this study.

Plasmids	Markers	Relevant properties	Source
pGEM-T easy	Amp	T7 and SP6 promoters	Promega
pG+host5	Ery	ColE1 replicon, thermosensitive derivative of pGK12	[9]
pTCV- <i>erm</i>	Ery, Km	Mob ⁺ (IncP); <i>oriR</i> pACYC184; <i>oriR</i> pAM_1	[10]
pTCVΩP _{cyl+} - <i>abx1</i>	Ery, Km	<i>abx1</i> complementing vector, promoter P _{abx1}	[8]
pG+host5ΩΔ <i>cylX</i>	Ery	<i>cylX</i> in-frame deletion vector	This study
pG+host5ΩΔ <i>cylD</i>	Ery	<i>cylD</i> in-frame deletion vector	This study
pG+host5ΩΔ <i>cylG</i>	Ery	<i>cylG</i> in-frame deletion vector	This study
pG+host5ΩΔ <i>cylAB</i>	Ery	<i>cylAB</i> in-frame deletion vector	This study
pG+host5ΩΔ <i>cylI</i>	Ery	<i>cylI</i> in-frame deletion vector	This study
pG+host5ΩΔIS	Ery	IS1381 in-frame deletion vector	This study
pG+host5ΩIS	Ery	Truncated IS1381 in-frame insertion vector	This study

Amp: Ampicillin; Ery: Erythromycin; Km: Kanamycin.

Table S4 : Primers used in this study.

Primer	Sequence (5'-3')*	Gene target
For plasmids constructions		
<i>cylX</i> -5b	aaaGGTACCaggcaagccattaatTTTTTgagt	Δ <i>cylX</i>
<i>cylX</i> -3b	aaaGGATCCcgtcatcaaagaaagaacggttgactt	Δ <i>cylX</i>
<i>cylX</i> -int1	ggcacgcccgggtgctgccgcctactcaaaatattagaacg	Δ <i>cylX</i>
<i>cylX</i> -int2	gcggcagcaccggggtgctgccctattaatggaaaggattgaa	Δ <i>cylX</i>
<i>cylAB</i> -5b	atctGAATTCatagtgggatatagatgaccaagc	Δ <i>cylAB</i>
<i>cylAB</i> -3b	acatGGATCCtgtcttatcatactctttgaagcca	Δ <i>cylAB</i>
<i>cylAB</i> -int1	aatgagggcaatgacataaatcaacttaattccattttctgataccaac	Δ <i>cylAB</i>
<i>cylAB</i> -int2	gttggtatcagaaaatggaaattaagttgatttatgcttgcctcattc	Δ <i>cylAB</i>
<i>cylD</i> -5b	aaaGAATTCtactcatgattttgtgaattcaac	Δ <i>cylD</i>
<i>cylD</i> -3b	aaaGGATCCtcatatctgtttcaatgaatc	Δ <i>cylD</i>
<i>cylD</i> -int1	ggcacgcccgggtgctgccgcctgaacattaccctgtcctcc	Δ <i>cylD</i>
<i>cylD</i> -int2	gcggcagcaccggggtgctgccagggggaggaaacagttatga	Δ <i>cylD</i>
<i>cylG</i> -5b	aaaGGTACCgtctgaagagctttggcttttg	Δ <i>cylG</i>
<i>cylG</i> -3b	aaaGGATCCcaatcatcaaaacaccgggaac	Δ <i>cylG</i>
<i>cylG</i> -int1	ggcacgcccgggtgctgccgcaccaccagtaactattgctac	Δ <i>cylG</i>
<i>cylG</i> -int2	gcggcagcaccggggtgctgccgtgattgatggaggaatgatt	Δ <i>cylG</i>
<i>cylI</i> -5b	acatGAATTCatcttgctagatttgTTTTctgg	Δ <i>cylI</i>
<i>cylI</i> -3b	acatGGATCCtgttccacgttttaacatttggtg	Δ <i>cylI</i>
<i>cylI</i> -int1	aataatagcaaaagagataccacctctattccactaacatatacgcctc	Δ <i>cylI</i>
<i>cylI</i> -int2	gagcgtatatgttagtggaataggaggtggtatctcttttgctattattg	Δ <i>cylI</i>
<i>cyl628</i> -EcoR1	gatatGAATTCgaagatgattagttgaaggtga	Δ <i>IS</i>
<i>cyl628</i> -Pst1	gacaaCTGCAGataagttgggcacgacgtt	Δ <i>IS</i>
<i>cyl628</i> -int1	ggatcccaagggaaggtaccaccaacaaggcgcttaaatcg	<i>cyl::IS</i>
<i>cyl628</i> -int2	ggtaccttcccttgggatcccctatcgaaatcgacgcaaacgg	<i>cyl::IS</i>
For sequence analysis		
<i>covR</i> _5'	ggttggggtagaagcggatttatcag	<i>covR</i>
<i>covR</i> _3'	ctttccatggattaccaatag	<i>covR</i>
<i>covS</i> _5'	gaaagatcagtttataggggtaaaac	<i>covS</i>
<i>covS</i> _3'	caaagcgcacataagtaatctc	<i>covS</i>
<i>covS2</i> _5'	tcagaagacgaaattggagaattg	<i>covS</i>
<i>abx1</i> -fwd	agtttctgcaactgaccttccac	<i>abx1</i>
<i>abx1</i> -rev	tgggcgataacttggtcattgtg	<i>abx1</i>
<i>stk1</i> -F	gctacagtactaacacaag	<i>stk1</i>
<i>stk1</i> -R	ctaggcatggtctctaccatagc	<i>stk1</i>
<i>stk1</i> -F2	gtaggtagtggtcattatt	<i>stk1</i>
<i>stk1</i> -F3	ctccaaacaagagtttcactc	<i>stk1</i>
<i>cylX</i> -5kon	cccgtgcttacttcttacaag	<i>cylX</i>
<i>cyl628</i> -5kon	gtgttgagaataatgcccctcatag	<i>cylX</i>
<i>cyl1</i> F	caacacttaagtcatgacctcatt	<i>cylX</i>
<i>cylD</i> -3kon	gcatcaatagagtctagctctaate	<i>cylD</i>
<i>cyl628</i> -3kon	gctactttcataactgtttcctcc	<i>cylD</i>
<i>cyl1.1</i> F	gttcatgcgtctatgttagc	<i>cylD</i>

cyl1.1R	ccatctgatgcaaattcttgc	<i>cylG</i>
cyl2F	gccctagaactgtcaataggga	<i>acpC</i>
cyl1R	ggaactataggacgttggtg	<i>cylZ</i>
cyl2.2F	cttggacctaattggagctggta	<i>cylA</i>
cyl2.3R	tcagctatcgaaagttggtc	<i>cylA</i>
cyl2.2R	gcaaagatgcaagagaacatcgtc	<i>cylB</i>
<i>cylEF</i>	gcgtaatgctgatggagaaaggct	<i>cylB</i>
cyl2R	aggcctaccgtctggaattga	<i>cylE</i>
<i>cylE.1F</i>	gtcgtagtggacaggcaatcactt	<i>cylE</i>
<i>cylE.1R</i>	cctccgatgatgcttgtaaacc	<i>cylE</i>
cyl4F	ggaactcaccttatgctacaaatg	<i>cylE</i>
<i>cylER</i>	ggaatcacaaggtctaagg	<i>cylF</i>
cyl4.1F	agcagagtatggggatgaactga	<i>cylF</i>
cyl4R	gcaggaatgtttctagagggtga	<i>cylI</i>
cyl4.1R	gctcctagtgatgtgaagcc	<i>cylI</i>
cyl5F	gcggcaatagaggaaacagactgta	<i>cylI</i>
cyl5.1F	tagtggagcgcttgatggta	<i>cylI</i>
cyl5R	gctgccatgcataaactgttcct	<i>cylJ</i>
cyl5.1R	gacggtaacctgtcaatacagc	<i>cylJ</i>
cyl6F	actgacaagctgtggtgaggctat	<i>cylJ</i>
<i>cylKbis</i>	cacatgttgcgagttagtattctgg	<i>cylK</i>
cyl6R	tcgctgatcttccggcgtgatatg	<i>gbs0657</i>
cyl7F	gatggcttatgctggactaacctc	<i>gbs0657</i>
cyl7R	aaccagacttcctcaggcttact	<i>gbs0658</i>

* Restriction sites are in capital letters.

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