

Supplementary information

Antibiotic resistance and pathogenicity assessment of various *Gardnerella* sp. strains in local China

Running title: **Antibiotic resistance and pathogenicity of *Gardnerella***

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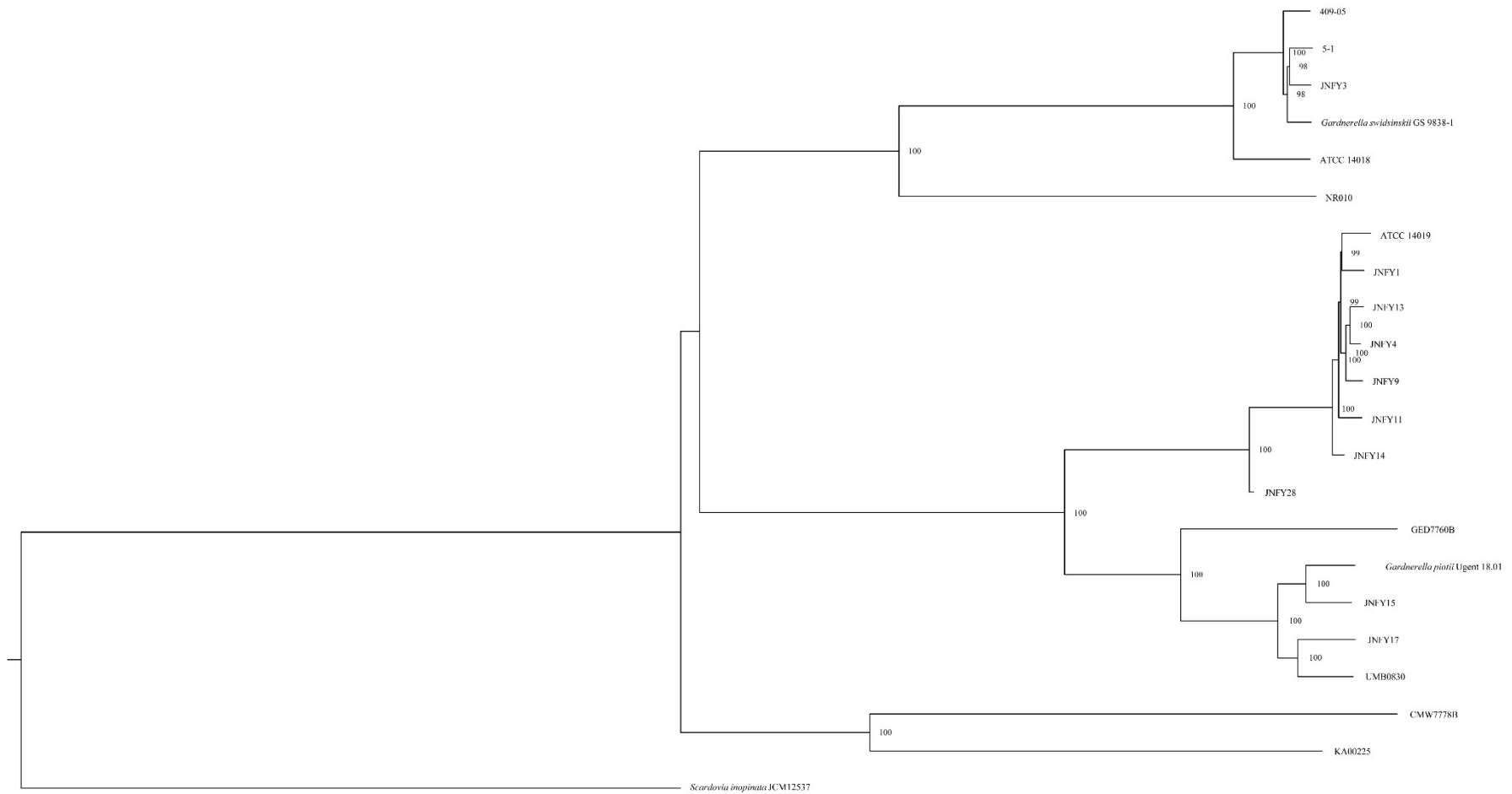
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I. Supplemental Figures

II. Supplemental Tables

I. Supplemental Figures

Figure S1. Phylogenomic tree of *Gardnerella* strains. A proteomic tree was constructed based on the genome sequences of 21 *Gardnerella* strains. The tree was built by the neighbor-joining method, and bootstrap value above 90 are shown at node points, based on 1,000 replicates.



0.03

II. Supplemental Tables

Table S1. PCR primers used in this study.

Primers	Sequence(5'-3')
16S27F	GAGTTTGATCCTGGCTCAG
16S1492R	AGAAAGGAGGTGATCCAGCC
cpn60F	ATGGCAAAGATTATTGCCTATGAAG
cpn60R	GCATTCTGTAGAGCAGAACGAGT
vlyF	GTACGATTCTGCAAGCGCACAAAGC
vlyR	CCTTCCCAAGCGGAGAACGC
sldF	ATGGAACGTCGTTCAACGAAG
sldR	GATACGCGTTTTATGTCTCTTGC

Table S2. Carbohydrate transport and metabolism genes comparison of the *Gardnerella* strains.

Gene	Annotation	ATCC 14019	JNFY1	JNFY3	JNFY4	JNFY9	JNFY11	JNFY13	JNFY14	JNFY15	JNFY17	JNFY28
AfuC	Alpha-L-fucosidase	1	1	0	1	1	1	1	1	0	0	0
AraD	Ribulose-5-phosphate 4-epimerase/Fuculose-1-phosphate aldolase	0	1	0	1	1	1	1	1	0	0	0
FucP	Fucose permease	0	1	0	1	1	1	1	1	0	0	0
GalM	Galactose mutarotase or related enzyme	0	1	0	1	1	1	1	1	0	0	0
GalT	Galactose-1-phosphate uridylyltransferase	2	1	0	1	1	1	1	1	0	0	0
LacZ	Beta-galactosidase/beta-glucuronidase	2	2	0	2	2	2	2	2	0	0	0
MglA	ABC-type sugar transport system, ATPase component	0	4	1	4	4	4	4	4	0	0	0
XylB	Sugar (pentulose or hexulose) kinase	0	2	0	2	2	2	2	2	0	0	0
XylF	ABC-type xylose transport system, periplasmic component	0	1	0	2	2	1	2	2	0	0	0
NagC	Sugar kinase of the NBD/HSP70 family, may contain an N-terminal HTH domain	0	7	1	8	7	8	8	7	4	2	2
UgpA	ABC-type sugar transport system, permease component	8	8	3	9	8	10	9	8	3	4	4
UgpB	ABC-type glycerol-3-phosphate	3	8	4	9	8	10	9	8	3	4	4

	transport component	system, periplasmic											
UgpE	ABC-type transport component	glycerol-3-phosphate system, permease	3	7	2	8	7	9	8	7	2	3	3

Table S3. Common virulence factors of the ten *Gardnerella* epidemic strains.

Virulence factor	Related gene	Product
Adhesion		
ABC transporter ATP-binding protein AatC	<i>aatC</i>	ABC transporter
Polyphosphate kinase(type IV pili)	<i>ppK</i>	polyphosphate kinase 1
Surface-anchored pilus proteins	<i>sapD</i>	actinobacterial surface-anchored protein domain
Elongation factor Tu	<i>tuf</i>	small GTP-binding protein domain
Trigger factor	<i>tig/ropA</i>	trigger factor
Immunogenic lipoprotein A(IlpA)	<i>IlpA</i>	NLPA lipoprotein
Listeria adhesion protein	<i>lap</i>	Aldehyde dehydrogenase family
Iron uptake		
Iron related(ABC transporter)	<i>fbpC</i>	ABC transporter
Putative iron transport system ATP-binding protein	<i>ciuD</i>	phosphate ABC transporter, ATP-binding protein
ABC transporter ATP-binding protein	<i>irtB</i>	FeS assembly ATPase SufC
Ferrous iron transport	<i>sitC</i>	anchored repeat-type ABC transporter, permease subunit
Transcriptional repressor of iron-responsive genes	<i>fur</i>	Ferric uptake regulator family
Iron(III)	<i>fbpC,hitC</i>	ABC transporter
Secretion system		
preprotein translocase subunit SecA (Accessory secretion factor)	<i>secA2</i>	preprotein translocase, SecA subunit
type III secretion system ATPase(T3SS)	<i>DVUA0119</i>	ATP synthase F1, alpha subunit
type III secretion system ATPase(T3SS)	<i>ssaN</i>	transcription termination factor Rho
Dot/Icm type IV secretion system effector	<i>lirB</i>	Cyclophilin type peptidyl-prolyl cis-trans

LirB(Dot/Icm) T6SS-II(CVF861)	protein disaggregation chaperone	isomerase ATP-dependent chaperone protein ClpB
Invasion		
Lipoprotein promoting entry protein P60 extracellular protein, invasion associated protein Iap	<i>lpeA</i> <i>iap/cwhA</i>	actinobacterial surface-anchored protein domain NlpC/P60 family
Stress protein		
endopeptidase Clp ATP-binding chain C(Clpc) ATP-dependent Clp protease proteolytic subunit(Clpp)	<i>clpC</i> <i>clpP</i>	ATPase family associated with various cellular Clp protease
Antibiotic resistance		
daunorubicin resistance ABC transporter ATP-binding subunit	<i>ddrA</i>	ABC transporter
Regulation of virulence-associated genes		
ppGpp synthetase, RelA/SpoT family S-ribosylhomocysteinase(Autoinducer-2)	<i>relA</i> <i>luxS</i>	RelA/SpoT family protein S-Ribosylhomocysteinase (LuxS)
two-component system response regulator(DevR/S)	<i>devR/dosR</i>	Response regulator receiver domain
membrane-bound transcriptional regulator LytR (Polysaccharide capsule)	<i>lytR</i>	cell envelope-related function transcriptional attenuator common domain

Table S4. Partially-shared virulence factors of the ten *Gardnerella* epidemic strains.

Virulence factor	Related gene	Product	Strains lacking the gene
Adhesion			
tight adherence protein A (Flp pili)	<i>tadA</i>	Type II/IV secretion system protein	JNFY3, JNFY13, JNFY28
SpaD-type pili	<i>srtB</i>	sortase	JNFY28
Type IV pili response regulator PilH	<i>pilH</i>	Response regulator receiver domain	JNFY15, JNFY17
Iron uptake			
phospho-2-dehydro-3-deoxyheptonate aldolase (vulnibactin)	<i>VVA1298</i>	3-deoxy-7-phosphoheptulonate synthase	JNFY3
Haemophilus iron transport locus	<i>hitC, hitC</i>	ABC transporter	JNFY3
putative iron ABC transport system,	<i>hmuV</i>	ABC transporter	JNFY3
Yersiniabactin (Ybt)	<i>ybtQ</i>	ABC transporter	JNFY3, JNFY9, JNFY15, JNFY17
Acinetobactin	<i>bauE, barA</i>	ABC transporter	JNFY3
Magnesium uptake			
Magnesium transport ATPase, P-type 2	<i>mgtB</i>	HAD ATPase, P-type, family IC	JNFY3
Secretion system			
ESX-1	<i>eccA1</i>	Holliday junction DNA helicase RuvB	JNFY3, JNFY15, JNFY17
Coxiella Dot/Icm type IVB secretion system translocated effector (Dot/Icm)	<i>coxH2, coxFIC1</i>	ribosomal-protein-alanine acetyltransferase	JNFY3, JNFY15
Type VII secretion system	<i>essC</i>	FtsK/SpoIIIE family	JNFY15

T6SS-III (CVF862)	periplasmic protein	sugar-binding	Periplasmic binding protein domain	JNFY15 、 JNFY17 、 JNFY28
Toxin				
vaginolysin	<i>HMPREF0424_0103</i>		Thiol-activated cytolysin	JNFY15
hemolysin A (Hemolysin)	<i>CLD_2757</i>		TlyA family rRNA methyltransferase	JNFY15、 JNFY17
HlyC/CorC family (Hemolysin)	<i>CLK_2301</i>		CBS domain	JNFY3
hemolysin B (Alpha-hemolysin)	<i>hlyB</i>		ABC transporter	JNFY15、 JNFY17
exo-alpha-sialidase (sialidase)	<i>nanJ, nanI</i>		BNR repeat-like domain	JNFY3
pyrroline-5-carboxylate reductase (Proline synthesis)	<i>proC</i>		pyrroline-5-carboxylate reductase	JNFY15
Endotoxin				
Fused lipid transporter subunits of ABC superfamily (LOS)	<i>msbA</i>		ABC transporter	JNFY3
Putative formyl transferase (LPS)	<i>wbmU</i>		methionyl-tRNA formyltransferase	JNFY15、 JNFY17
Immune vasion				
lipooligosaccharide biosynthesis galactosyltransferase, putative (LOS)	<i>CJE1280</i>		Glycosyl transferase family 2	JNFY3、 JNFY15、 JNFY17
phosphoglucosamine mutase (Exopolysaccharide)	<i>mrsA/glmM</i>		phosphoglucosamine mutase	JNFY3
exopolysaccharide biosynthesis protein, glycosyl transferase	<i>eps15</i>		Glycosyl transferases group 1	JNFY3、 JNFY15、 JNFY17

(Capsule)			
RgpG (Capsule)	<i>SGO_1723</i>	phospho-N-acetylmuramoyl-pentapeptide-transferase	JNFY3、JNFY15、JNFY17
dTDP-4-dehydrorhamnose 3,5-epimerase or related enzyme	<i>STER_1223</i>	dTDP-4-dehydrorhamnose 3,5-epimerase	JNFY3
(Capsule)			
Regulation			
lysozyme	<i>lytC</i>	Glycosyl hydrolases family 25	JNFY15 、 JNFY17 、 JNFY28
WhiB family transcriptional regulator (WhiB3)	<i>whiB3</i>	Transcription factor WhiB	JNFY3
RNA polymerase sigma factor SigA (Sigma A)	<i>sigA/rpoV</i>	RNA polymerase sigma factor RpoD	JNFY15、JNFY17
DNA-binding response regulator PhoP (PhoP/R)	<i>phoP</i>	Response regulator receiver domain	JNFY3
two-component system response phosphate sensor kinase, PhoR (PhoP/R)	<i>phoR</i>	His Kinase A (phospho-acceptor) domain	JNFY3 、 JNFY13 、 JNFY15、JNFY17
two component transcriptional regulator (RegX3)	<i>regX3</i>	Response regulator receiver domain	JNFY3
GTP pyrophosphokinase (RelA)	<i>relA</i>	Region found in RelA / SpoT proteins	JNFY3

Table S5. Unique virulence factors of strains JNFY3 and JNFY4.

Strain	Virulence factor	Related gene
JNFY3	allantoate amidohydrolase (Allantion utilization)	<i>allC</i>
	Allantoinase (Allantion utilization)	<i>allB</i>
	Glycosyltransferase (Capsule I)	<i>BCAL3234</i>
	putative polysaccharide permease protein (Capsule)	<i>SGO_1023</i>
	choline permease (LOS)	<i>licB</i>
	aggregation substance (AS)	<i>EF0149</i>
JNFY4	Bartonella effector protein BepA (VirB/VirD4 type IV secretion system)	<i>bepA</i>