The ISME Journal (2021) 15:2158–2162
https://doi.org/10.1038/s41396-021-00934-w

BRIEF COMMUNICATION

Conserved bacterial de novo guanine biosynthesis pathway enables microbial survival and colonization in the environmental niche of the urinary tract

Deepak S. Ipe[1,2] · Matthew J. Sullivan[1] · Kelvin G. K. Goh[1] · Saeed M. Hashimi[2] · Alan L. Munn[1] · Glen C. Ulett[1]

Received: 27 November 2020 / Accepted: 8 February 2021 / Published online: 1 March 2021

Abstract

In bacteria, *guaA* encodes guanosine monophosphate synthetase that confers an ability to biosynthesize guanine nucleotides de novo. This enables bacterial colonization in different environments and, while *guaA* is widely distributed among *Bacteroidetes* and *Firmicutes*, its contribution to the inhabitation of the human microbiome by commensal bacteria is unclear. We studied *Streptococcus* as a commensal urogenital tract bacterium and opportunistic pathogen, and explored the role of *guaA* in bacterial survival and colonization of urine. Analysis of *guaA*-deficient *Streptococcus* revealed guanine utilization is essential for bacterial colonization of this niche. The genomic location of *guaA* in other commensals of the human urogenital tract revealed substantial cross-phyla diversity and organizational structures of *guaA* that are divergent across phyla. Essentiality of *guaA* for *Streptococcus* colonization in the urinary tract establishes that purine biosynthesis is a critical element of the ability of this bacterium to survive and colonize in the host as part of the resident human microbiome.

All bacterial taxa require purine nucleotides to accomplish essential metabolic processes such as the synthesis of DNA, RNA, and protein. Several pathways for de novo purine synthesis and salvage converge to satisfy this requirement in bacteria and thereby, support survival in niche environments that are limited in purine bioavailability, as reported for *Borrelia* [1], *Erwinia* [2], *Lactococcus* [3], and *Helicobacter* [4]. Phosphoribosyl pyrophosphate is utilized to generate purines, and the biosynthetic pathway bifurcates at the point of inosine monophosphate (IMP) to produce adenine monophosphate or guanine monophosphate (GMP) as precursors for deoxyribonucleotides. The latter requires *guaA*-encoded GMP synthetase, which provides Guanosine-5′-triphosphate for essential cellular processes.

The resident microbiota of the human microbiome inhabits diverse niches that vary in nutrient availability [5]. One such niche is the "urogenital tract", a term which refers to an organ system that encompasses the urinary tract as well as the anatomical sites/organs of reproduction, which may affect the microbial load in urine, as reviewed elsewhere in the context of the human microbiome [6]. Different tissues of the live human host vary in relative concentrations of purines which are influenced by external factors such as diet [7]. In human urine (HU), the purine guanine is a precursor of uric acid and potential biomarker of disease [8]. Bacteria of the commensal microbiome persist in the urogenital tract through metabolic flexibility [6]; however, the extent to which purines such as guanine are utilized remains largely unclear. Among the estimated 50 genera of the human urogenital microbiota [6], several including *Streptococcus* persist as commensals but can also act as opportunistic pathogens. We explored the role of *guaA* in *Streptococcus* survival and colonization in the urinary tract as a part of the "urogenital tract" niche.

We analyzed the growth of wild-type (WT) *S. agalactiae* 834 compared to a *guaA*-deficient mutant (Supplementary

These authors contributed equally: Deepak S. Ipe, Matthew J. Sullivan

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41396-021-00934-w.

✉ Glen C. Ulett
g.ulett@griffith.edu.au

[1] School of Medical Science, and Menzies Health Institute Queensland, Griffith University, Parklands, QLD, Australia

[2] Present address: School of Dentistry and Oral Health, and Menzies Health Institute Queensland, Griffith University, Parklands, QLD, Australia

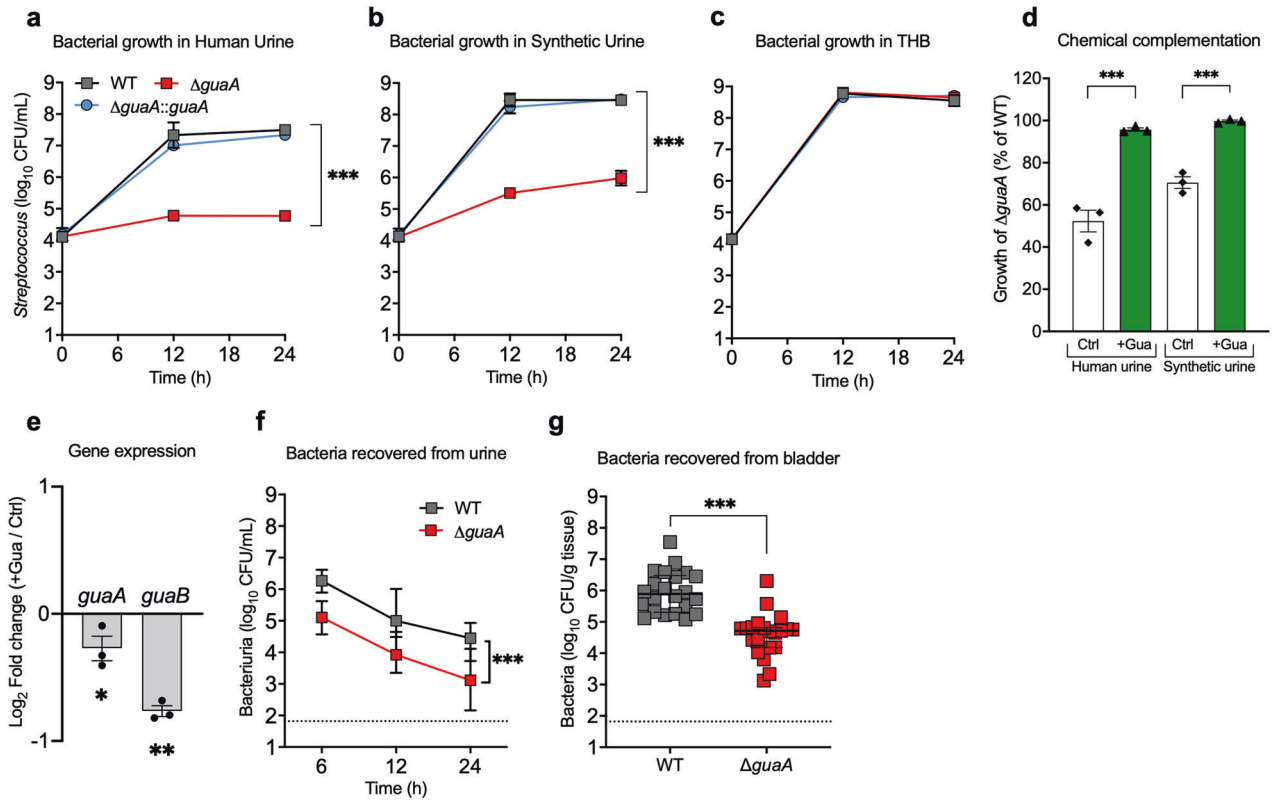

Fig. 1 *guaA* supports *S. agalactiae* growth in urine and colonization of the mouse bladder. a Growth of WT and *guaA*-deficient *S. agalactiae* were compared in human urine (HU), **b** synthetic human urine (SHU), and **c** THB with *guaA* supplied in trans to complement the mutation (Δ*guaA*::*guaA*). **d** WT and Δ*guaA S. agalactiae* grown in the absence (Ctrl) or presence of 0.5 mM guanine-HCl (+Gua), compared in the same growth conditions at the 24 h timepoint. **e** Expression (fold change) of *guaA* and *guaB* was quantified from WT *S. agalactiae* in SHU ± 0.5 mM guanine. Data shown are pooled results of three to five independent assays, each using different batches of HU and SHU; bars show means ± SEM, compared using area under the curve and Student's *t* tests. The number of bacteria recovered from the (**f**) urine, and (**g**) bladders of female C57BL/6 mice at 24 h post-inoculation. Data shown are from three independent experiments, each with ≥7 mice per group. Data were compared using Mann–Whitney *U* test (***$p < 0.001$). The bars represent medians, displayed with interquartile ranges.

Table S1) in HU pooled from several individuals. We also compared the growth of these strains in synthetic human urine, which revealed a striking attenuation of *guaA*$^-$ *S. agalactiae* in both conditions (Fig. 1a, b). Parallel growth assays using Todd Hewitt Broth showed no general growth defect of the *guaA* mutant (Fig. 1c). Owing to its contribution to GMP synthesis, we were able to chemically complement the *guaA*$^-$ mutation by supplying exogenous guanine, confirming that *guaA* is essential to support bacterial survival and growth in environments where guanine levels are limited (Fig. 1d). Consistent with our findings, *guaA* supports survival of *E. coli* in urine, which has been associated with a shift to commensalism for this gram-negative organism [9–11]. We note the genomic arrangement of *guaA* and *guaB* (encoding IMP dehydrogenase that catalyzes the step of GMP synthesis that precedes that of *guaA*) in *Streptococcus* differs markedly from *E. coli* (Fig. S1) and *Staphylococcus* in which control systems of these genes are well-defined [12, 13]. Transcriptional analyses showed modest repression of *Streptococcus guaA* and *guaB* in media conditions of exogenous guanine (Fig. 1e), despite the distinct genomic arrangement; consistent with findings in *E. coli* in which *guaA* is not induced as a milieu-specific response following culture in urine [11]. Together, these observations hint at convergent systems of guanine-dependent control of *guaA* regardless of spatial gene arrangement disparities among members of the commensal urogenital tract microbiota. Interestingly, some strains of uropathogenic *Escherichia coli* upregulate *guaA* only during active UTI but not during growth in pooled HU ex vivo [11]; these findings suggest that there may be value in examining the transcriptional activity of the *gua* genes in the context of active *S. agalactiae* UTI in the host environment.

Analysis of the role of *guaA* in bacterial colonization in vivo revealed a marked attenuation in survival of *guaA*$^-$ *Streptococcus* compared to the WT in urine of mice that were experimentally infected by transurethral delivery of bacteria to the bladder (Fig. 1f). A similar attenuation was detected in the bladders 24 h after infection (Fig. 1g),

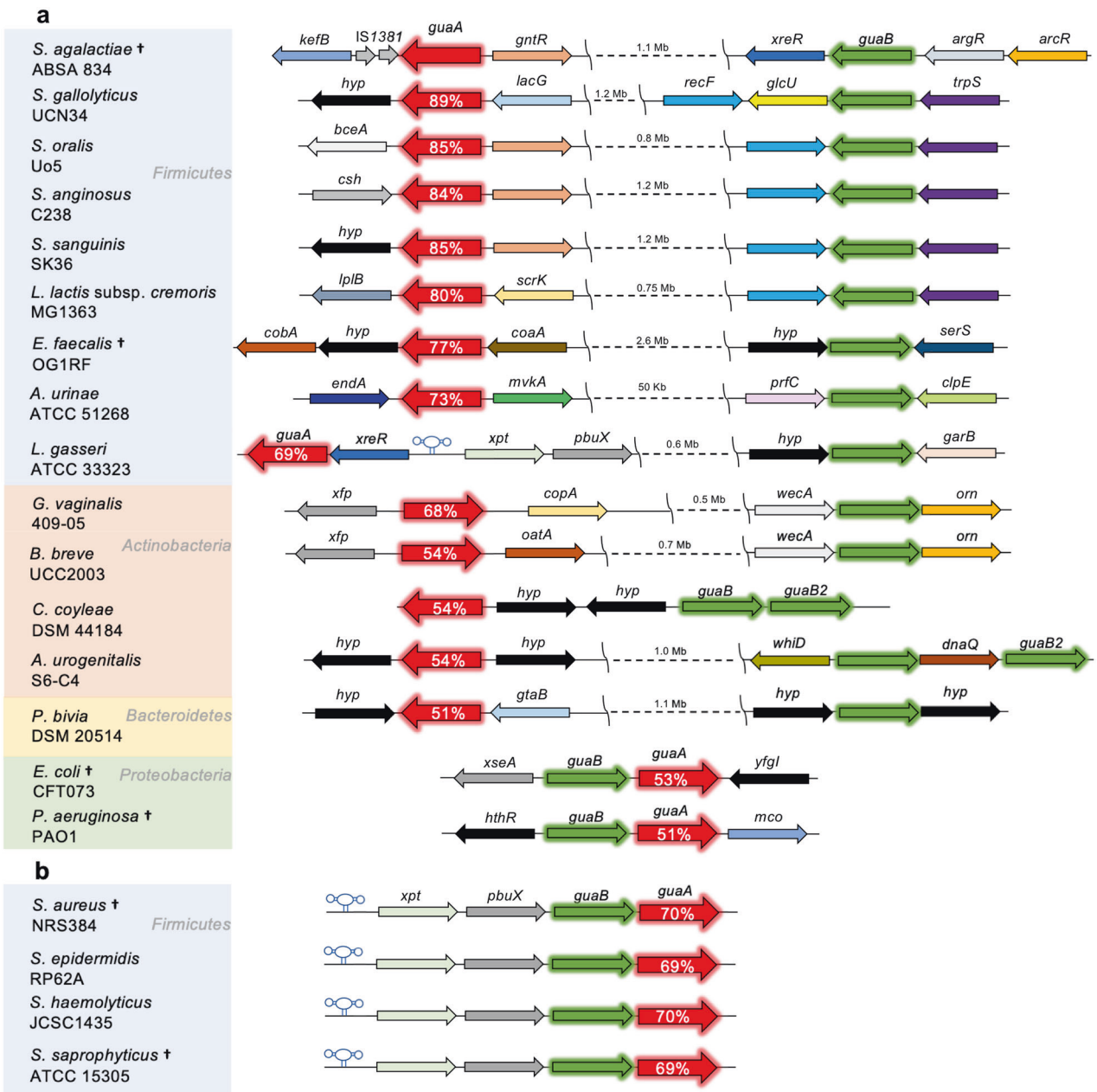

Fig. 2 Cross-phyla genetic organization and sequence diversity of *guaA* among commensal bacterial genera of the human urogenital tract microbiota. a Genomic arrangement of *guaA* and *guaB* homologs in different bacterial species that colonize the urogenital tract niche [23], using reference genomes of representative members of selected genera for which sequence data are available. Where *guaA* (red) and *guaB* (green) are separated, the number of bases between the genes is indicated by dashed lines. In *S. agalactiae* 834, *IS*1381 insertion element is located at the 3′ end of *guaA*, which is often co-located divergently from a GntR-type regulator in streptococci. Percentage identity to *S. agalactiae guaA* is shown in white text. †bacterial species for which some members are uropathogenic and cause UTI [31]. **b** In staphylococci, *guaA* and *guaB* are adjacent to purine salvage genes *xpt* and *pbuX*, and are controlled by an upstream guanine-sensing riboswitch [12].

illuminating a major role for *guaA* in mediating *Streptococcus* survival and colonization in the urinary tract. Bacterial adaptation to this niche may represent an evolutionary strategy toward commensalism [14]; the finding of *guaA*'s contribution to *S. agalactiae* survival and colonization in the urinary tract may offer new explanation of how other gram-positive bacteria survive and persist in the urinary tract, given a high degree of conservation in the sequence of *guaA* among clinical isolates (Supplementary Table S2). Additional mechanisms that underpin bacterial survival in urine include acquisition of iron, malic acid metabolism, and tolerance to D-serine [15]. Notably, *Streptococcus guaA* also contributes to bacterial survival in other body niches, including blood [16]; this is relevant given that *S.*

agalactiae can spread haematogenously to cause pyelonephritis [17] and can also cause urosepsis [18]. A recent clinical study reported that urine was the second most common source for *S. agalactiae* bacteraemia [19]. In this context, it is noteworthy that mammalian plasma contains very low concentrations of purines and purine nucleosides, which can vary during states of physiological stress and disease [20].

In *Firmicutes*, including *S. agalactiae*, *guaA* is a hotspot for mobile insertion elements [21, 22], offering potential for genomic rearrangement at this locus. We analyzed the genomic locations of *guaA* in streptococci and other commensal bacteria of the human urogenital tract [23] to reveal substantial cross-phyla diversity among *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, and *Proteobacteria*, and an organizational structure that is distinct from the *guaAB* operon characteristic of many enteric bacteria and staphylococci (Fig. 2) [12, 13]. Despite relatively conserved amino acid sequences among the *Firmicutes* (62–89% homology) analyzed in this study, *guaA* genetic organization as well as sequence diversity is more apparent comparing between other phyla (Figs. 2 and S1). Additional elements of gene regulation and gene transfer are also apparent, including the presence of guanine riboswitch sequences that effect transcriptional activation and insertion sequences that reflect the hotspot for integration.

The discovery that *guaA* is critical for survival of and colonization by bacteria in urine aligns closely to studies of nutritional immunity that show metabolic gene products can be crucial to microbial fitness in a host. The ability of microbes to persist and proliferate in host niches depends on essential nutrient provision, which can stem from the host or be synthesized de novo. A widely studied example is iron acquisition and the counteraction of iron limitation by the host to restrict microbial growth, which might be harnessed to medicinally target bacteria [24]. Only certain nutrients are limiting in urine [10] and guanine is one of these for different types of bacteria. More broadly, previous studies that have identified selective inhibitors of guanine-related metabolic pathways have used such compounds to treat infection [25]. Interestingly, a prior study demonstrated that infection of the mouse bladder with *S. agalactiae* 834 engages the innate immune response to generate an inflammatory, chemotactic, and regulatory cytokine milieu [26]. Thus, at least in mice, the host significantly activates immune defenses in response to this bacterium, which was isolated from the urine of an asymptomatic adult [27, 28]. Some patterns of cytokine production in mice with acute UTI parallel immune responses that occur in humans with UTI, as reviewed elsewhere [29]; however, reconciling the asymptomatic nature of *S. agalactiae* 834 as a clinical isolate with stimulation of innate immune responses in mice will require further study.

Some bacteria, such as *E. coli* can behave as a commensal organisms in some host niches such as the gut, but not in other host niches such as the urinary tract (where the bacteria cause inflammation and persists in the absence of antibiotic treatment) and the vagina [30]. Further understanding of *S. agalactiae* as a commensal of the human urogenital tract and an opportunistic pathogen will require analysis of the bacteria's requirements for survival in different host niches. It will be of interest to study the genetic organization of *guaA* and other genes (for guanine salvage), the regulatory mechanisms of their activities in relevant models of infection, and characterize the role of metabolic gene products, including guanine as potential targets for microbial control. A proposed pathway for guanine metabolism in *S. agalactiae* is illustrated in Supplementary Fig. S2. Work on other commensal microbes should examine guanine utilization in host niches, given the diverse environmental and host tissue habitats that these microbes encounter.

Acknowledgements This work was supported by funding from a Griffith University New Researcher Grant (MSC GUNRG 219152; to DSI), the National Health and Medical Research Council (Project Grant APP1146820; to GCU), the Griffith Health Institute and a Future Fellowship from the Australian Research Council (FT110101048; to GCU). The authors thank Harry Sakelaris and James A. Fraser for helpful discussions.

Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

1. Jewett MW, Lawrence KA, Bestor A, Byram R, Gherardini F, Rosa PA. GuaA and GuaB are essential for *Borrelia burgdorferi* survival in the tick-mouse infection cycle. J Bacteriol. 2009;191:6231–41.
2. Eastgate JA, Thompson L, Milner J, Cooper RM, Pollitt CE, Roberts IS. Identification of a nonpathogenic *Erwinia amylovora guaB* mutant. Plant Pathol. 1997;46:594–9.
3. Duwat P, Ehrlich SD, Gruss A. Effects of metabolic flux on stress response pathways in *Lactococcus lactis*. Mol Microbiol. 1999;31:845–58.
4. Liechti G, Goldberg JB. *Helicobacter pylori* relies primarily on the purine salvage pathway for purine nucleotide biosynthesis. J Bacteriol. 2012;194:839–54.
5. Costello EK, Lauber CL, Hamady M, Fierer N, Gordon JI, Knight R. Bacterial community variation in human body habitats across space and time. Science. 2009;326:1694–7.
6. Neugent ML, Hulyalkar NV, Nguyen VH, Zimmern PE, De Nisco NJ. Advances in understanding the human urinary microbiome and its potential role in urinary tract infection. mBio. 2020;11:pii: mBio.00218–20.
7. Chitty JL, Fraser JA. Purine acquisition and synthesis by human fungal pathogens. Microorganisms. 2017;5:pii: microorganisms 5020033.

8. Maiuolo J, Oppedisano F, Gratteri S, Muscoli C, Mollace V. Regulation of uric acid metabolism and excretion. Int J Cardiol. 2016;213:8–14.
9. Hull RA, Hull SI. Nutritional requirements for growth of uropathogenic *Escherichia coli* in human urine. Infect Immun. 1997;65:1960–1.
10. Russo TA, Jodush ST, Brown JJ, Johnson JR. Identification of two previously unrecognized genes (*guaA* and *argC*) important for uropathogenesis. Mol Microbiol. 1996;22:217–29.
11. Subashchandrabose S, Hazen TH, Brumbaugh AR, Himpsl SD, Smith SN, Ernst RD, et al. Host-specific induction of *Escherichia coli* fitness genes during human urinary tract infection. Proc Natl Acad Sci USA. 2014;111:18327–32.
12. Kofoed EM, Yan D, Katakam AK, Reichelt M, Lin B, Kim J, et al. De Novo guanine biosynthesis but not the riboswitch-regulated purine salvage pathway is required for *Staphylococcus aureus* infection in vivo. J Bacteriol. 2016;198:2001–15.
13. Mehra RK, Drabble WT. Dual control of the gua operon of *Escherichia coli* K12 by adenine and guanine nucleotides. J Gen Microbiol. 1981;123:27–37.
14. Dobrindt U, Wullt B, Svanborg C. Asymtomatic bacteriuria as a model to study the coevolution of hosts and bacteria. Pathogens. 2016;5:pii: E21.
15. Ipe DS, Horton E, Ulett GC. The basics of bacteriuria: strategies of microbes for persistence in urine. Front Cell Infect Microbiol. 2016;6:14.
16. Jones AL, Knoll KM, Rubens CE. Identification of *Streptococcus agalactiae* virulence genes in the neonatal rat sepsis model using signature-tagged mutagenesis. Mol Microbiol. 2000;37:1444–55.
17. Sullivan MJ, Ulett GC. Evaluation of hematogenous spread and ascending infection in the pathogenesis of acute pyelonephritis due to group B streptococcus in mice. Micro Pathog. 2020;138:103796.
18. Freudenstein D, Reinshagen K, Petzold A, Debus A, Schroten H, Tenenbaum T. Ultra late onset group B streptococcal sepsis with acute renal failure in a child with urethral obstruction: a case report. J Med Case Rep. 2012;6:68.
19. Alizzi M, Rathnayake R, Sivabalan P, Emeto TI, Norton R. Group B streptococcal bacteraemia—changing trends in a tropical region of Australia. Intern Med J. 2020. https://doi.org/10.1111/imj.15164.
20. McFarland WC, Stocker BA. Effect of different purine auxotrophic mutations on mouse-virulence of a Vi-positive strain of Salmonella dublin and of two strains of *Salmonella typhimurium*. Micro Pathog. 1987;3:129–41.
21. Song L, Pan Y, Chen S, Zhang X. Structural characteristics of genomic islands associated with GMP synthases as integration hotspot among sequenced microbial genomes. Comput Biol Chem. 2012;36:62–70.
22. Brochet M, Rusniok C, Couve E, Dramsi S, Poyart C, Trieu-Cuot P, et al. Shaping a bacterial genome by large chromosomal replacements, the evolutionary history of Streptococcus agalactiae. Proc Natl Acad Sci USA. 2008;105:15961–6.
23. Hilt EE, McKinley K, Pearce MM, Rosenfeld AB, Zilliox MJ, Mueller ER, et al. Urine is not sterile: use of enhanced urine culture techniques to detect resident bacterial flora in the adult female bladder. J Clin Microbiol. 2014;52:871–6.
24. Sargun A, Gerner RR, Raffatellu M, Nolan EM. Harnessing iron acquisition machinery to target *Enterobacteriaceae*. J Infect Dis. 2020:pii: 6039829. https://doi.org/10.1093/infdis/jiaa440.
25. Mulhbacher J, Brouillette E, Allard M, Fortier LC, Malouin F, Lafontaine DA. Novel riboswitch ligand analogs as selective inhibitors of guanine-related metabolic pathways. PLoS Pathog. 2010;6:e1000865.
26. Leclercq SY, Sullivan MJ, Ipe DS, Smith JP, Cripps AW, Ulett GC. Pathogenesis of *Streptococcus* urinary tract infection depends on bacterial strain and beta-hemolysin/cytolysin that mediates cytotoxicity, cytokine synthesis, inflammation and virulence. Sci Rep. 2016;6:29000.
27. Ipe DS, Ben Zakour NL, Sullivan MJ, Beatson SA, Ulett KB, Benjamin WHJ, et al. Discovery and characterization of human urine utilization by asymptomatic-bacteriuria-causing *Streptococcus agalactiae*. Infect Immun. 2015;84:307–19.
28. Ulett KB, Benjamin WH Jr., Zhuo F, Xiao M, Kong F, Gilbert GL, et al. Diversity of group B streptococcus serotypes causing urinary tract infection in adults. J Clin Microbiol. 2009;47:2055–60.
29. Carey AJ, Tan CK, Ipe DS, Sullivan MJ, Cripps AW, Schembri MA, et al. Urinary tract infection of mice to model human disease: practicalities, implications and limitations. Crit Rev Microbiol. 2016;42:780–99.
30. Brannon JR, Dunigan TL, Beebout CJ, Ross T, Wiebe MA, Reynolds WS, et al. Invasion of vaginal epithelial cells by uropathogenic Escherichia coli. Nat Commun. 2020;11:2803.
31. Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nat Rev Microbiol. 2015;13:269–84.