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***Streptococcus pyogenes* (“Group A Streptococcus”), a Highly Adapted Human Pathogen—Potential Implications of Its Virulence Regulation for Epidemiology and Disease Management**

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Streptococcus pyogenes (group A streptococci; GAS) is an exclusively human pathogen which can cause a wide range of suppurative and nonsuppurative diseases in people of all ages worldwide. Not all can be successfully treated with antibiotics and in spite of its global importance, there is not yet a licensed vaccine available. GAS infections are complex and multifactorial processes, and both host and bacteria factors are crucial for successful establishment of an infection. GAS expresses an arsenal of virulence factors responsible for pathological immune reactions. The transcription of all these virulence factors is under the control of three types of virulence-related regulators: (i) two-component systems (TCS), (ii) stand-alone regulators, and (iii) non-coding RNAs.

In this review, the authors focus on the regulation of established virulence factors of GAS, which play a major role in the different diseases caused by this versatile pathogen. They summarize major TCS and stand-alone transcriptional regulatory systems: CovR/S, Ihk/Irr, CiaH/R, FasBCA/X, multiple gene regulator of group A Streptococci-Mga, RLAP Family Stand-Alone Regulators, Regg/RopB and PerR; and discussed their potential as antibacterial targets. It is suggested that this better knowledge on the genetics of virulence regulation should be use for the development of new therapies and prevention methods for GAS infections

***Streptococcus pyogenes* (“estreptococo del grupo A”), un patógeno humano altamente adaptado: posibles implicaciones de su regulación de virulencia para la epidemiología y el manejo de enfermedades**

Streptococcus pyogenes (estreptococo del grupo A; GAS por su sigla en inglés) es un patógeno exclusivamente humano, responsable de una variedad de enfermedades supurativas y no supurativas en personas de todas las edades en todo el mundo. No todas las infecciones causadas por este microorganismo pueden tratarse exitosamente con antibióticos y a pesar de su gran importancia, todavía no existe una vacuna disponible. Las infecciones por GAS son procesos complejos y multifactoriales, y tanto los factores del huésped como de las bacterias son cruciales para el

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establecimiento exitoso de una infección. Los GAS expresan un arsenal de factores de virulencia responsables de las reacciones inmunes patológicas. La transcripción de todos estos factores de virulencia está bajo el control de tres tipos de reguladores relacionados con la virulencia: (i) sistemas de dos componentes (TCS), (ii) reguladores transcripcionales independientes y (iii) ARN no codificantes. Los autores de esta revisión se centran en los factores de virulencia de GAS y su regulación, que están directamente asociados con el control de la virulencia y juegan un papel principal en las diferentes enfermedades causadas por este patógeno tan versátil. Se centraron en los principales TCS y reguladores transcripcionales independientes: CovR/S, Ihk/Irr, CiaH/R, FasBCA/X, Mga (una proteína reguladora de múltiples genes del grupo A Streptococci, los reguladores transcripcionales independientes de la familia RLAP, Regg/RopB y PerR; y analizan su potencial como dianas antibacterianas. Se sugiere que este mayor conocimiento sobre la genética de la regulación de la virulencia debería utilizarse para el desarrollo de nuevas terapias y métodos de prevención para las infecciones por GAS.

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Changes in the incidence of invasive disease due to *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Neisseria meningitidis* during the COVID-19 pandemic in 26 countries and territories in the Invasive Respiratory Infection Surveillance Initiative: a prospective analysis of surveillance data.

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Streptococcus pneumoniae, *Haemophilus influenzae*, and *Neisseria meningitidis*, which are generally transmitted through respiratory droplets, are the main causes of invasive diseases, such as pneumonia and bacteremic meningitis, as well as secondary infections after a viral respiratory illness. In this article, the authors assess the incidence of these invasive bacterial infections during the COVID-19 pandemic and the previous two years. Surveillance data were collected between January 1, 2018 and May 31, 2020. Weekly case numbers in 2020 were compared to 2018 and 2019. Data were also collected from nine laboratories on invasive disease due to *Streptococcus agalactiae*, a non-respiratory pathogen. The stringency of the COVID-19 containment measures was quantified using the Oxford Government COVID-19 Response Tracker. Changes in population movements were assessed using Google's COVID-19 Community Mobility Reports. An interrupted time series model was used to assess changes in incidence. Twenty-seven laboratories submitted data to IRIS for *S. pneumoniae* (62837 cases in total), 24 laboratories from 24 countries submitted data for *H. influenzae* (7796 cases in total), and 21 laboratories from 21 countries submitted data for *N. meningitidis* (5877 cases in total). All countries had experienced a significant and sustained reduction in invasive diseases caused by these bacteria in early 2020, compared to the previous 2 years. The authors found no evidence of changes in *S. agalactiae* presentations in 2020 compared to 2018 and 2019, supporting the view that reductions in *S. pneumoniae*, *H. influenzae*, and *N. meningitidis* cases in 2020 were due to the decrease in the incidence of the disease. Similar trends were observed in most countries and territories. The investigators found that the incidence of reported *S. pneumoniae* infections decreased by 68% at 4 weeks (incidence rate index 0.32 [95% CI 0.27 to 0.37]) and 82% at 8 weeks (0.18 [0.14-0.23]) when there were significant changes in population movements. The reductions in the weeks after the expected interruption of the time series were strongly supported by a mixed effects negative binomial distribution model, $p < 0.0001$. Although the stringency of the containment measures imposed varied by country, there was a pronounced reduction in notifications of invasive *S. pneumoniae* disease in all participating countries, and this reduction was maintained until the end of May 2020. The introduction of COVID-19 containment policies and public information campaigns likely reduced the transmission of *S. pneumoniae*, *H. influenzae*, and *N. meningitidis*, leading to a significant reduction in life-threatening invasive diseases in many countries across the world. It is highlighted as a limitation of the study that the countries participating in IRIS are high- and middle-income countries, which could reduce the generalizability of the results; however, reducing the respiratory transmission of bacteria will reduce the incidence of invasive diseases, something beneficial in any country.

Cambios en la incidencia de enfermedad invasiva debida a *Streptococcus pneumoniae*, *Haemophilus influenzae* y *Neisseria meningitidis* durante la pandemia de COVID-19 en 26 países y territorios en la Iniciativa de Vigilancia de

Infecciones Respiratorias Invasivas: un análisis prospectivo de los datos de vigilancia

Streptococcus pneumoniae, *Haemophilus influenzae* y *Neisseria meningitidis*, que generalmente se transmiten a través de gotitas respiratorias, son las principales causas de enfermedades invasivas, como neumonía y meningitis bacteriémica, así como infecciones secundarias después de una enfermedad respiratoria viral. En este artículo, los autores evalúan la incidencia de estas infecciones bacterianas invasivas durante la pandemia de COVID-19 y los dos años anteriores.

Se recabaron datos de la vigilancia entre el 1 de enero de 2018 y el 31 de mayo de 2020. Los números de casos semanales en 2020 se compararon con 2018 y 2019. También se recopilaron datos de nueve laboratorios sobre la enfermedad invasiva debida a *Streptococcus agalactiae*, un patógeno no respiratorio. La rigurosidad de las medidas de contención de COVID-19 se cuantificó utilizando el Rastreador de Respuesta COVID-19 del Gobierno de Oxford. Los cambios en los movimientos de población se evaluaron mediante los informes de movilidad comunitaria COVID-19 de Google. Se utilizó un modelo de series de tiempo interrumpido para evaluar los cambios en la incidencia.

Veintisiete laboratorios enviaron datos a IRIS para *S. pneumoniae* (62.837 casos en total), 24 laboratorios de 24 países enviaron datos para *H. influenzae* (7.796 casos en total) y 21 laboratorios de 21 países enviaron datos para *N. meningitidis* (5.877 casos en total). Todos los países habían experimentado una reducción significativa y sostenida de las enfermedades invasivas causadas por estas bacterias a principios de 2020, en comparación con los 2 años anteriores. Los autores no encontraron evidencia de cambios en las presentaciones de *S. agalactiae* en 2020 en comparación con 2018 y 2019, lo que respalda la opinión de que las reducciones en los casos de *S. pneumoniae*, *H. influenzae* y *N. meningitidis* en 2020 se debieron a la disminución en la incidencia de la enfermedad. Se observaron tendencias similares en la mayoría de los países y territorios. Los investigadores encontraron que la incidencia de infecciones por *S. pneumoniae* notificadas disminuyó en un 68% a las 4 semanas (índice de tasa de incidencia 0,32 [IC del 95%: 0,27 a 0,37]) y un 82% a las 8 semanas (0,18 [0,14-0,23]) cuando hubo cambios significativos en los movimientos de población. Las reducciones en las semanas posteriores a la interrupción esperada de la serie temporal fueron fuertemente apoyadas por un modelo de distribución binomial negativa de efectos mixtos, $p < 0,0001$). Aunque el rigor de las medidas de contención impuestas varió según el país, hubo una reducción pronunciada en las notificaciones de la enfermedad invasiva por *S. pneumoniae* en todos los países participantes, y esta reducción se mantuvo hasta finales de mayo de 2020.

La introducción de políticas de contención de COVID-19 y campañas de información pública probablemente redujeron la transmisión de estas bacterias, lo que llevó a una reducción significativa de enfermedades invasivas potencialmente mortales en muchos países de todo el mundo. Se destaca como limitación del estudio, que los países que participan en IRIS son países de ingresos altos y medios, lo que podría incidir en la

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generalización de los resultados; sin embargo, la reducción de la transmisión respiratoria de bacterias reducirá la incidencia de enfermedades invasivas, algo beneficioso en cualquier país.

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Development and Clinical Evaluation of a CRISPR-Based Diagnostic for Rapid Group B Streptococcus Screening

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Vertical transmission of group B Streptococcus (GBS) is among the leading causes of neonatal illness and death. GBS carriage is often intermittent, and the rate of GBS colonization varies during pregnancy. Colonization with GBS usually is screened weeks before delivery during pregnancy. Determination of colonization at the time of delivery is crucial for the prevention of neonatal infection. Author of the article developed CRISPR-GBS, a novel CRISPR/Cas13-based in vitro diagnostic assay, and conducted a prospective cohort study and a validation study in >400 clinical cases to evaluate its diagnostic performance among different technology platforms, including culture techniques and PCR-based methods. Performed analytical study and after passing this, evaluated the diagnostic potential of CRISPR-GBS in settings of clinical screening. A total of 412 pregnant women whose sample collection was performed at 34–38 weeks of gestation were tested for GBS by culture, PCR, and CRISPR-GBS on their direct swab samples. Authors found no significant differences between patients who were negative or positive for GBS on the basis of patient age or weeks of gestation, greater sensitivity of CRISPR and suggested that the testing direct swabs by CRISPR-GBS conferred comparable sensitivity as enrichment culture.

Authors also compared turnaround time, and found that the CRISPR-GBS test required an average of <1.5 hours, which includes 30 minutes of rapid DNA extraction, 30 minutes for DNA amplification by RPA, and 20 minutes for Cas13 detection, which is a considerable advantage over those for conventional culture-based (24–60 hours) and PCR-based (≈2.5 hours for a regular PCR assay and much longer for nested PCR–Sanger) methods. The important issue to consider is that authors used fluorescence detection, but according to other manuscripts, this technique could be adapted to simplest-colorimetric detection (Group Strep comment) <https://doi.org/10.3389/fmolb.2020.582499>.

Authors also mention the possibility of improved this technology to detect antimicrobial resistance genes direct- from-swab testing, that would permit the precise approach to

identification of GBS colonization and prevention of related neonatal diseases.

Desarrollo y evaluación clínica de un diagnóstico basado en CRISPR para la detección rápida de estreptococos del grupo B

La transmisión vertical de Estreptococos del grupo B (GBS) es una de las principales causas de enfermedad y muerte neonatal. La colonización por GBS es intermitente y su rango varía durante el embarazo. El tamizaje prenatal en general ocurre algunas semanas antes del término del embarazo y es por ello que en ciertas ocasiones, un resultado negativo no garantiza la ausencia de GBS en el momento del parto. Por tal motivo, se requieren nuevos y mejores métodos de detección de la colonización por GBS durante el parto. Los autores de este artículo desarrollaron un sistema de detección basado en la tecnología CRISPR-Cas13 para detectar GBS. Para testear la sensibilidad y especificidad de este sistema validaron el test con más de 400 muestras clínicas y compararon los resultados con los métodos convencionales de cultivo, enriquecimiento y PCR específica. Luego de la prueba analítica exitosa, los autores utilizaron este test en 412 mujeres a partir de muestras tomadas entre la semana 34 y 38 de embarazo donde testean la presencia de GBS por cultivo, PCR y CRISPR-GBS a partir de las muestras (hisopados). No se encontraron diferencias entre pacientes que fueron positivos o negativos para GBS en relación a la edad y período de gestación. De nuevo, detectaron similar sensibilidad entre CRISPR y el cultivo.

El método desarrollado acorta los tiempos requeridos a 1,5 h donde 30 minutos involucran la extracción de ADN, 30 minutos la amplificación y 20 minutos la detección por CAS13, comparada con las 24-60 h de cultivo, y las 2,5h de PCR. Es importante mencionar que los autores utilizan como método de detección, la fluorescencia, y que esto a futuro puede adaptarse a un sistema colorimétrico (comentario de Grupo Strep). <https://doi.org/10.3389/fmolb.2020.582499>. Los autores además mencionan que en un futuro podrían utilizar esta tecnología para detectar genes de resistencia a antibióticos directamente del hisopo, lo que permitiría realizar la identificación y prevención de la enfermedad por GBS.