UNIVERSIDADE ESTADUAL DE MARINGÁ
CENTRO DE CIÊNCIAS DA SAÚDE
DEPARTAMENTO DE ANÁLISES CLÍNICAS E BIOMEDICINA
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOCIÊNCIAS E FISIOPATOLOGIA

LINCOLN LUÍS SILVA

Avaliação dos recursos de saúde para o enfretamento de doenças infecciosas utilizando ferramentas geoespaciais

Maringá
2023

LINCOLN LUÍS SILVA

Avaliação dos recursos de saúde para o enfretamento de doenças infecciosas utilizando ferramentas geoespaciais

Tese apresentada ao Programa de Pós-Graduação em Biociências e Fisiopatologia do Departamento de Análises Clínicas e Biomedicina, Centro de Ciências da Saúde da Universidade Estadual de Maringá, como requisito parcial para obtenção do título de Doutor em Biociências e Fisiopatologia

Área de concentração: Biociências e Fisiopatologia Aplicadas à Farmácia

Orientadora: Profa. Dra. Rosilene Fressatti Cardoso

Co-Orientador: Prof. Dr. João Ricardo Nickenig Vissoci

Maringá

2023

Dados Internacionais de Catalogação-na-Publicação (CIP)
(Biblioteca Central - UEM, Maringá - PR, Brasil)

S586a

Silva, Lincoln Luís

Avaliação dos recursos de saúde para o enfretamento de doenças infecciosas utilizando ferramentas geoespaciais / Lincoln Luís Silva. -- Maringá, PR, 2023.

113 f.: il. color., figs., tabs., maps.

Orientadora: Profa. Dra. Rosilene Fressatti Cardoso.

Coorientador: Prof. Dr. João Ricardo Nickenig Vissoci.

Tese (Doutorado) - Universidade Estadual de Maringá, Centro de Ciências da Saúde, Departamento de Análises Clínicas e Biomedicina, Programa de Pós-Graduação em Biociências e Fisiopatologia (PBF), 2023.

1. Covid-19. 2. Ferramentas geoepaciais. 3. Análise espacial. 4. Epidemiologia. 5. Saúde pública. I. Cardoso, Rosilene Fressatti , orient. II. Vissoci, João Ricardo Nickenig , coorient. III. Universidade Estadual de Maringá. Centro de Ciências da Saúde. Departamento de Análises Clínicas e Biomedicina. Programa de Pós-Graduação em Biociências e Fisiopatologia (PBF). IV. Título.

CDD 23.ed. 614.4

Rosana de Souza Costa de Oliveira - 9/1366

FOLHA DE APROVAÇÃO

Lincoln Luís Silva

"Avaliação dos recursos de saúde para o enfrentamento de doenças infecciosas utilizando ferramentas geoespaciais",

Tese apresentada ao Programa de Pós-Graduação em Biociências e Fisiopatologia do Departamento de Análises Clínicas e Biomedicina, Centro de Ciências da Saúde da Universidade Estadual de Maringá, como requisito parcial para obtenção do título de Doutor em Biociências e Fisiopatologia pela Comissão Julgadora composta pelos membros:

COMISSÃO JULGADORA

Prof.ª Dr.ª Rosilene Fressatti Cardoso
Universidade Estadual de Maringá (Presidente)

Dr. Luciano Andrade
UEM – Membro

Dr.ª Sandra Maria Pelloso
UEM – membro

Dr.ª Raissa Bocchi Pedroso
UNINGÁ – Membro

Dr. Thiago Augusto Hernandes Rocha
PAHO – membro
Participação Remota

Aprovada em: 17 de fevereiro de 2023
Local de Defesa: sala 112 B, bloco T20 - Universidade Estadual de Maringá

DEDICATÓRIAS

Dedico este trabalho a todos aqueles que contribuíram para sua realização.

AGRADECIMENTOS

Primeiramente gostaria de agradecer a Deus pelo dom da vida.

À minha noiva, Amanda Gubert, pelo amor, carinho, incentivo, companheirismo, compreensão e paciência em todos os momentos durante essa caminhada.

Aos meus pais, Solange e Luís, pelo amor, apoio e compreensão.

Aos meus avós Marlene e Milton, por estarem comigo para dividir todos os momentos.

Aos meus amigos Adnan, Mateus, Bruna, Cinthia, Regivaldo, Isabella, e Amanda de Carvalho pela amizade, companheirismo e compreensão.

À minha orientadora Profa. Dra. Rosilene Fressatti Cardoso por ter aceitado me ajudar nessa etapa da minha vida, e por quem tenho admiração.

Ao meu coorientador Prof Dr. Joao Ricardo Nickenig Vissoci por ter aceitado participar no desenvolvimento desse trabalho, por ter me recebido na Duke University, pelo apoio, pela parceria, e amizade.

À Profa. Dra. Sandra Marisa Pelloso pelo apoio, incentivo e por ter me apresentado um outro lado da pesquisa.

Ao Professor e amigo Luciano de Andrade pela parceria e inspiração.

Ao Professor e amigo Thiago Augusto Hernandes Rocha pela ajuda com assuntos profissionais e pessoais, e por ser um exemplo para mim.

Ao Prof. Dr. Wuelton Marcelo Monteiro pela parceria nos seus trabalhos que serviram de inspiração para o desenvolvimento do segundo artigo da tese.

À Vânia, secretária do Programa de Pós-Graduação em Biociências e Fisiopatologia (PBF), por ter me ajudado com as questões burocráticas.

Ao PBF pela capacitação a nível de doutorado.

À Universidade Estadual de Maringá, por ter me proporcionado ingressar na graduação e pós-graduação.

À Duke University, onde fui muito bem recebido para desenvolver parte do meu doutorado.

A todos que contribuíram diretamente e/ou indiretamente na minha formação.

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Código de Financiamento 001

EPÍGRAFE

“Educação não transforma o mundo.
Educação muda as pessoas.
Pessoas transformam o mundo.”
- Paulo Freire

Avaliação dos recursos de saúde para o enfretamento de doenças infecciosas utilizando ferramentas geoespaciais

Resumo

A pandemia do COVID-19 teve um impacto significativo no sistema de saúde global, e o Brasil não é exceção. No primeiro artigo, o objetivo foi verificar o preparo do Sistema de Saúde Brasileiro para a pandemia. Portanto, um estudo retrospectivo e ecológico foi realizado com dados do Departamento Brasileiro de Informática do Sistema Único de Saúde. O estudo analisou os números de leitos de terapia intensiva (UTI) e hospitalares, médicos gerais ou intensivistas, enfermeiros, técnicos de enfermagem, fisioterapeutas e ventiladores de cada região de saúde por meio da área de captação flutuante de 2 degraus, análise espacial que verifica a acessibilidade ao cuidado. Além disso, outra análise espacial usando Getis-Ord-Gi* também foi realizada para detectar hotspots de alta incidência de COVID-19 e distribuição de leitos hospitalares. Os resultados revelaram que, em fevereiro de 2020, o Brasil tinha 35.682 leitos de UTI, 426.388 leitos hospitalares e 65.411 ventiladores. Além disso, 17.240 novos leitos de UTI foram criados em junho de 2020. No entanto, a distribuição desses recursos foi desigual entre as regiões, com as regiões Sul e Sudeste apresentando os maiores índices de profissionais e infraestrutura para atender pacientes com COVID-19 em comparação com a região Norte região. A região Norte apresentou a menor acessibilidade às UTIs. O estudo concluiu que o Sistema de Saúde brasileiro estava inadequadamente preparado para gerir a pandemia devido à distribuição desigual de unidades de saúde, equipamentos e recursos humanos e à ineficácia das medidas públicas das administrações municipal e federal. No segundo trabalho, outro estudo foi realizado no Brasil para fornecer informações sobre como otimizar a distribuição de vacinas COVID-19 usando a análise de localização-alocação, uma análise espacial que verifica o número ideal de instalações necessárias para maximizar a cobertura. Este estudo coletou dados do Sistema de Informação do Programa Nacional de Imunizações em uma população do Sul do Brasil para determinar o número de pessoas vacinadas e a localização dos locais de vacinação. A localização de todos os estabelecimentos de saúde autorizados a administrar vacinas foi obtida no Registro Nacional de Estabelecimentos de Saúde para identificar possíveis novos locais. O estudo constatou que, até dezembro de 2021, quase todos os municípios do estado atingiram uma taxa de imunização de 70% e identificaram 56 novos estabelecimentos em potencial que aumentariam a cobertura populacional em mais de

250 mil pessoas. No geral, o estudo descobriu que a utilização de técnicas de alocação de localização pode ajudar os formuladores de políticas a garantir a distribuição adequada das vacinas COVID-19. No terceiro trabalho, foi realizado um estudo na Carolina do Norte, Estados Unidos da América, para identificar áreas com diferentes níveis de Determinantes Sociais da Saúde (SDOH) afetados desproporcionalmente pela COVID-19 em termos de taxas de infecção, hospitalização e mortalidade. O estudo analisou infecções por COVID-19 e SDOH em seis condados de Durham, Chatham, Granville, Orange, Person e Wake, na Carolina do Norte, observando os dados do paciente do registro de um sistema de saúde de março de 2020 a fevereiro de 2021 de um determinado hospital. Além disso, um conjunto de 14 variáveis SDOH de cada setor censitário para cada município foi coletado do American Community Survey 1 e 5-Year Data em 2020, e posterior redução dimensional por análise de componentes principais para criar três grupos de vulnerabilidade social. Esses grupos foram, então, inseridos na análise multivariada de agrupamento para identificar clusters de vulnerabilidade social. Em seguida, foi realizada uma estimativa de densidade de kernel para verificar os hotspots de pacientes com COVID-19 sobrepostos a esses clusters para verificar se a vulnerabilidade e as áreas com grande número de casos estavam relacionadas. Por fim, o Kruskal-Wallis foi realizado para verificar a proporção de casos entre clusters para confirmar a relação entre vulnerabilidade e número de casos. O estudo descobriu que aglomerados de disparidades sociais aumentadas foram significativamente mais afetados pelo COVID-19 do que áreas com melhor SDOH. Em conclusão, os formuladores de políticas devem promover ações para melhorar as condições de vida das pessoas mais vulneráveis para garantir que possam ter um melhor resultado contra o COVID-19.

Palavras-chave: COVID-19. SARS-CoV-2. Spatial analysis. Epidemiology. Public health. Social Determinants of Health. Vaccine. Access to Health Care.

Evaluation of health resources for coping with infectious diseases using geospatial tools

ABSTRACT

The COVID-19 pandemic has had a significant impact on the global health system, and Brazil is no exception. In the first article, the objective was to verify the preparedness of the Brazilian Health Care System for the pandemic. Therefore, a retrospective and ecological study was conducted using data from the Brazilian Information Technology Department of the Public Health Care System. The study analyzed the numbers of intensive care (ICU) and hospital beds, general or intensivist physicians, nurses, nursing technicians, physiotherapists, and ventilators from each health region using 2-step floating catchment area, a spatial analysis that verifies the accessibility to care. In addition, another spatial analysis using Getis-Ord-Gi* was also performed in to detect hotspots of high incidence of COVID-19 and distribution of hospital beds. The results revealed that, as of February 2020, Brazil had 35,682 ICU beds, 426,388 hospital beds, and 65,411 ventilators. In addition, 17,240 new ICU beds were created in June 2020. However, the distribution of these resources was uneven across regions, with the South and Southeast regions having the highest rates of professionals and infrastructure to attend patients with COVID-19 compared to the northern region. The northern region had the lowest accessibility to ICUs. The study concluded that the Brazilian Health Care System was inadequately prepared to manage the pandemic due to the inequitable distribution of health facilities, equipment, and human resources, and the ineffectiveness of public measures of the municipal and federal administrations. In the second work, another study was conducted in Brazil to provide information on how to optimize the distribution of COVID-19 vaccines using location-allocation analysis, a spatial analysis that verifies the optimal number of facilities needed to maximize coverage. This study collected data from the National Immunization Program Information System in in a population in Southern Brazil to determine the number of people vaccinated and the location of vaccination sites. The location of all health facilities authorized to administer vaccines was obtained from the National registry of health establishments to identify potential new sites. The study found that by December 2021, nearly all municipalities in the state reached an immunization rate of 70% and identified 56 new potential establishments that would increase population coverage by more than 250,000 people. Overall, the study found that utilizing location-allocation techniques can assist policy makers

in ensuring proper distribution of COVID-19 vaccines. In the third work, a study in North Carolina, United States of America, was conducted to identify areas with different levels of Social Determinants of Health (SDOH) were disproportionately affected by COVID-19 in terms of infection, hospitalization, and death rates. The study analyzed COVID-19 infections and SDOH in six counties Durham, Chatham, Granville, Orange, Person, and Wake in North Carolina by looking at patient data from a healthcare system's registry from March 2020 to February 2021 from a particular hospital. In addition, a set of 14 SDOH variables of each census-tract for each county were collected from the American Community Survey 1 and 5-Year Data in 2020, and posterior dimensional reduction by principal components analysis to create three groups of social vulnerability. These groups then were inserted to multivariate clustering analysis to identify clusters of social vulnerability. Next, a kernel density estimation was performed to verify the hotspots of patients with COVID-19 overlapping these clusters to verify whether the vulnerability and areas with great number of cases were related. Lastly, Kruskal-Wallis was performed to verify the proportion of cases between clusters to confirm the relationship between vulnerability and number of cases. The study found that clusters of increased social disparities was significantly more affected by COVID-19 than areas with better SDOH. In conclusion, policy makers must promote actions to improve the living conditions of the most vulnerable people to ensure that they can have a better outcome against COVID-19.

Keywords: COVID-19. SARS-CoV-2. Spatial Analysis. Epidemiology. Public health. Social Determinants of Health. Vaccine. Access to Health Care.

LISTA DE ILUSTRAÇÕES

LISTA DE TABELAS

Tese elaborada e formatada conforme as normas das publicações científicas: Frontiers in Public Health (artigo 1) disponível em: https://www.frontiersin.org/journals/public-health/for-authors/author-guidelines, e Plos One (artigos 2 e 3) disponível em: https://journals.plos.org/plosone/s/submission-guidelines.

SUMÁRIO

CAPÍTULO I

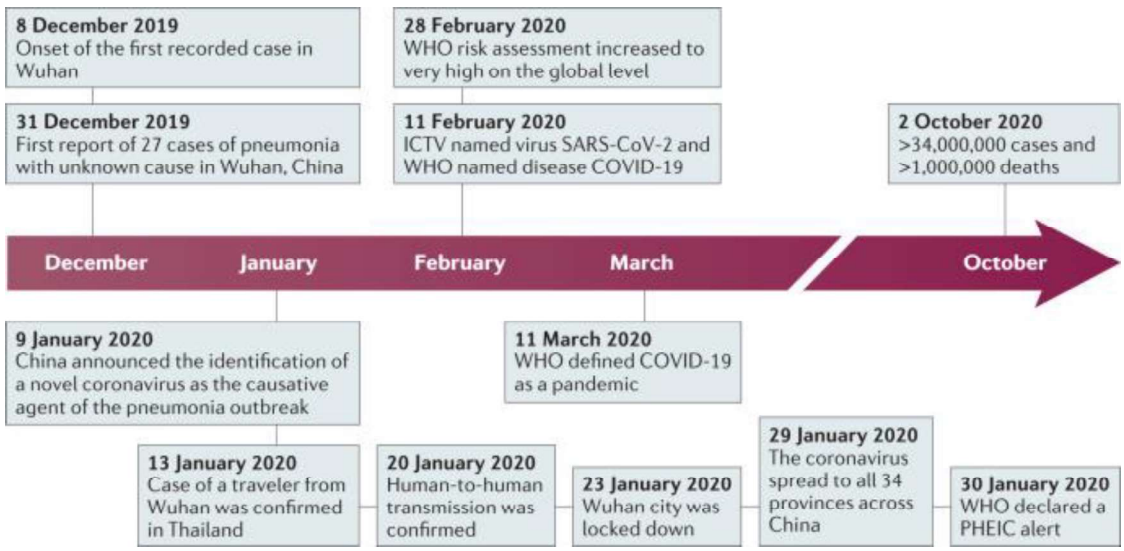
INTRODUÇÃO

HISTÓRICO

A doença viral Coronavírus de 2019 (COVID-19) causada pelo coronavírus 2 da síndrome respiratória aguda grave (SARS-CoV-2) resultou numa pandemia que ruiu o sistema de saúde de diversos países do mundo onde milhões de pessoas adoeceram e morreram (1).

Antes da identificação do agente etiológico, inicialmente houve um surto de uma doença respiratória em que os pacientes foram diagnosticados com pneumonia de origem desconhecida, onde os primeiros casos foram identificados província de Wuhan, China, em dezembro de 2019 (Figura 1) (2). Ainda no mesmo mês, as autoridades chinesas notificaram a Organização Mundial de Saúde (OMS) sobre o ocorrido e o Centro de Controle e Prevenção de Doenças (CDC) da China afirmou que a causa desse surto foi devido à um novo coronavírus com mais de 70% de homologia com o SARS-CoV e mais de 95% de semelhança com o coronavírus de morcego (3).

Figura 1 - Evolução temporal dos eventos da COVID-19, desde a identificação até a declaração de pandemia.



Fonte: Hu, Ben, et al. (2020) (4).

Em janeiro de 2021 o Comitê Internacional de Taxonomia de Vírus nomeou esse vírus como coronavírus da síndrome respiratória aguda grave 2 (SARS-CoV-2), e a OMS nomeou a doença como COVID-19 (5), além de declarar a COVID-19 como uma crise de saúde internacional quando mais de 9.000 pacientes foram diagnosticados em todo o mundo(6). As autoridades de saúde verificaram que esse vírus possuía alta capacidade de transmissibilidade e patogenicidade, e que como consequência rapidamente se espalhou pelos continentes, se tornando um problema de saúde pública global até que finalmente em 11 de março de 2021, a OMS declarou a COVID-19 como pandêmica (7).

Enquanto a COVID-19 avançava da Asia para a Europa e América do Norte com aumento de milhares de casos diariamente (8), na América Latina o primeiro caso da doença foi identificado no Brasil, em São Paulo, região sudeste do país (9, 10). Embora a COVID-19 tenha chegado tardiamente nas américas, já era motivo de grande preocupação uma vez que historicamente o sistema de saúde dos países que compõem a América Latina são demasiadamente frágeis (11), pois já eram assolados por outras doenças como tuberculose, malária e sarampo (12). Além disso, já era de se esperar que o impacto da COVID-19 seria ainda mais devastador em países com baixos indicadores socioeconômicos e estrutura governamental instável como do Caribe e Venezuela do que nas economias mais desenvolvidas, como Brasil (13).

No Brasil, o primeiro caso foi de um homem de 61 anos, que viajou de 9 a 20 de fevereiro de 2020 para a Lombardia, região norte da Itália, onde estava correndo um surto significativo da COVID-19 (13). Retornou ao Brasil no dia 21 de fevereiro de 2020 e foi atendido no Hospital Albert Einstein em São Paulo, onde foi diagnosticado com a doença (13). O Brasil era referência mundial no combate às grandes ameaças infecciosas, como HIV, tuberculose e malária, e que, portanto, gerou uma grande expectativa para verificar seu preparo frente a COVID-19 (14). Contudo, como o país passava por um conturbado período político que afetou gravemente o sistema de saúde, o país se tornou o epicentro da epidemia na América Latina (15).

AGENTE ETIOLÓGICO

O SARS-CoV-2 é um vírus de RNA de sentido positivo (+ssRNA) de fita simples, pertencente à linhagem B do gênero Beta-coronavírus da família *Coronaviridae* (16), capaz de infectar diferentes tipos de animais, incluindo os humanos, levando a doenças respiratórias de gravidade leve a grave (4). Sob aspecto genômico, SARS-CoV-2 compartilha cerca de 79% do

genoma do coronavírus da síndrome respiratória aguda grave (SARS-CoV) e 50% do coronavírus da síndrome respiratória do Oriente Médio (MERS-COV), dois outros vírus altamente patogênicos de origem zoonótica, que entre 2002 a 2012 surgiram em humanos e causaram doenças respiratórias fatais, tornando os coronavírus emergentes uma nova preocupação de saúde pública logo no início do século 21 (17).

Quanto a estrutura do SARS-CoV-2, é basicamente composta pelas quatro proteínas estruturais: a proteína do nucleocapsídeo (N) que forma o capsídeo fora do genoma, o qual é ainda empacotado por um envelope constituído pela proteína de membrana (M), proteína *spike* (S) e proteína do envelope (E), além de conter mais dezesseis proteínas não estruturais (*nsp*1-16) (18). A proteína S é, em especial, constituída por peptídeo sinal (SP), domínio de ligação ao receptor (RBD), subdomínio 1 (SD1) e subdomínio 2 (SD2) na subunidade S1 e peptídeo de fusão (FP), repetição heptad 1 (HR1), repetição heptad 2 (HR2) e transmembrana (TM) na subunidade de fusão de membrana (S2), importantes proteínas envolvidas no mecanismo de patogenia da doença (19).

Apesar da semelhança com outros coronavírus, existem outras cepas de SARS-CoV-2 com sutis diferenças nas sequências genéticas. De acordo com o Novel Coronavirus Resource of China National Center for Bioinformation, após avaliar 77.801 sequências genômicas de SARS-CoV-2 detectadas globalmente, foi identificado um total de 15.018 mutações (4), sendo que 13 ocorreram na proteína *spike*, conferindo a mutação D614G a cepa mais infecciosa identificada no início da pandemia (18).

Após a comparação genômica, verificou-se quanto a origem do vírus. Os resultados indicam uma identidade de 96,2% do SARS-CoV-2 com a cepa de coronavírus do tipo SARS de morcego BatCov RaTG13 (20). Esses estudos sugerem que o SARS-CoV-2 pode ser de origem do morcego, sendo um dos reservatórios naturais do SARS-CoV-2 (18).

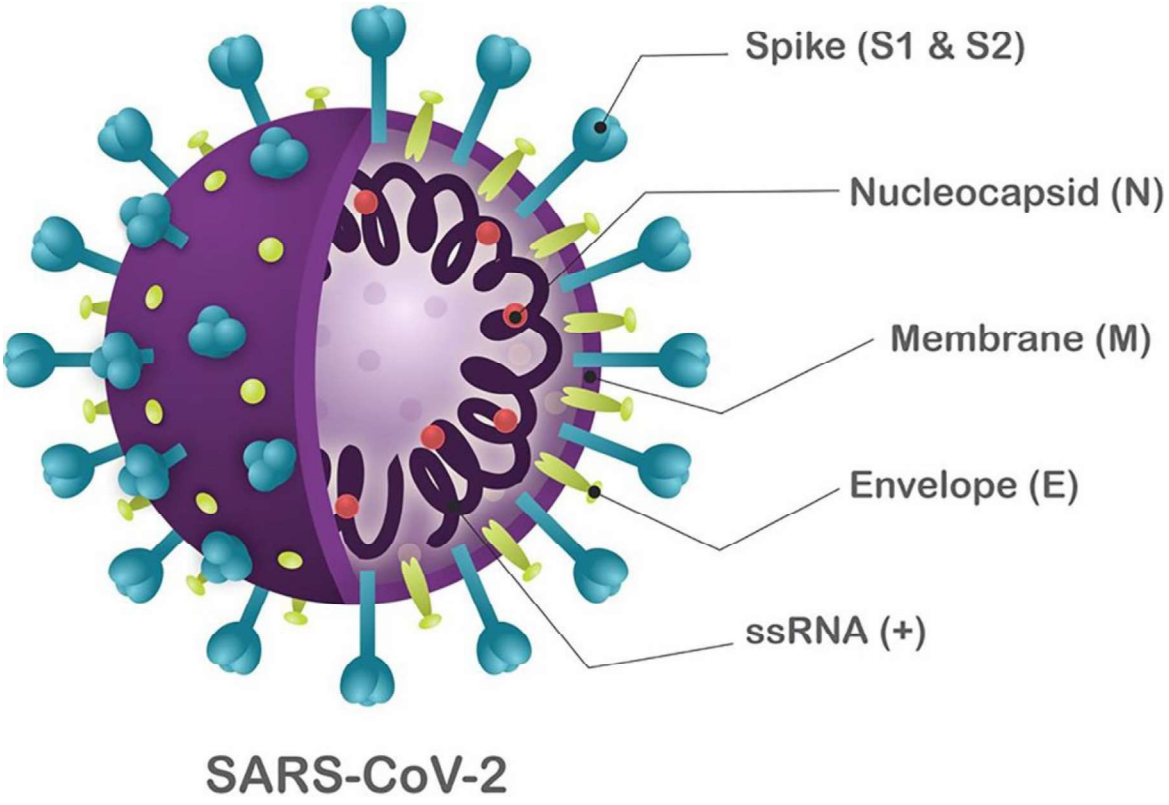
PATOGENIA

O SARS-CoV-2 é transmitido via respiratória por gotículas durante o contato próximo e desprotegido entre infectados e não infectados por meio da fala, tosse e espirros (18). O vírus também pode se espalhar por transmissão indireta, isto é, gotículas contendo vírus contaminam os indivíduos por meio de objetos, quando estes de alguma maneira entram em contato com as membranas mucosas da boca, nariz e olhos (18).

Quanto ao mecanismo de ação, SARS-CoV-2 entra na célula hospedeira pela ligação do receptor RBD da proteína S aos receptores de superfície da célula hospedeira humana (19),

majoritariamente pela a enzima conversora de angiotensina 2 (ACE2), uma metaloprotease amplamente expressa nas células do pulmão, o principal sitio de infecção da COVID-19, mas também pode acometer o intestino, fígado, coração, endotélio vascular, testículo e rim (21). Após a interação RBD-receptor, a proteína S sofre clivagem proteolítica, permitindo a fusão da membrana viral-hospedeiro, seguida pela liberação do RNA viral no citoplasma do hospedeiro (22). No citoplasma, o RNA viral utiliza o maquinário do hospedeiro para replicar seu material genético e montar novas partículas virais (Figura 2) (19).

Figura 2 - Estrutura do SARS-CoV-2



Fonte: Santos, et al. (2020) (23).

O início dos sintomas ocorre em média 5 a 6 dias a partir da exposição e, normalmente, aqueles com sintomas leves se recuperam em 2 semanas; entretanto, em casos graves, a recuperação pode se estender por até 6 semanas (24). Outros estudos ainda verificaram que o período de recuperação é independente da severidade pois os sintomas podem persistir ou recorrer por semanas ou meses após a aparente recuperação inicial (25). Contudo, contrariamente a esses estudos, existem pessoas que não apresentaram sintoma algum no percurso da infecção (26).

Os principais sintomas da COVID-19 são semelhantes a algumas infecções respiratórias causadas por vírus (27). A grande maioria dos pacientes sintomáticos geralmente apresenta febre, tosse e falta de ar e, menos comumente, dor de garganta, anosmia, disgeusia, anorexia, náusea, mal-estar, e mialgias (28). Sintomas gastrointestinais como diarreia, náusea e vômito também foram relatados, mas com frequências mais baixas, e a perda de olfato ou paladar varia significativamente entre os estudos também foram relatados (29). Embora a transmissão aérea seja predominante na COVID-19, SARS-CoV-2 também foi detectado nas fezes de alguns pacientes (30). Isso explica o surgimento de sintomas gastrointestinais em algumas pessoas com COVID-19 (31). Entretanto, manifestações clínicas mais preocupantes também foram verificadas como: síndrome da insuficiência respiratória aguda, hipóxia, hipotensão, problemas cardíacos, danos no sistema nervoso central, tromboembolismo e outros (29).

Por fim, a classificação dos casos dos pacientes pôde ser determinada com base nos sintomas relatados mundialmente, a qual é a mesma classificação utilizada pelo Brasil, dividida em: a) assintomático; b) leve; c) moderado; d) grave; e) crítico (32), e está apresentada no quadro abaixo.

TRATAMENTO

Durante a fase inicial da pandemia, pacientes com quadros mais brandos da COVID-19 eram recomendados a ficar em casa e fazer uso de medicamentos antigripais uma vez que os sintomas eram semelhantes aos da gripe comum (33, 34). Contudo, pacientes com casos mais graves, principalmente aqueles institucionalizados, tinham um complexo uso de medicamento como utilização de antiinflamatórios esteroidais, podendo ser combinada com monoclonais e outras terapias alternativas.

A dexametasona é um corticosteroide utilizado como antiinflamatório e muito promissor no tratamento da COVID-19 (35). A dexametasona leva a uma diminuição nas taxas de mortalidade, especialmente em pacientes gravemente enfermos necessitando de suporte ventilatório ou necessitando de oxigenoterapia. O uso de interferon também foi amplamente utilizada nas unidades de terapia intensiva como forma de aumentar a resposta imunológica dos pacientes pois estudos anteriores à COVID-19 demonstraram que SARS-CoV e MERS-CoV interrompia a cadeia de sinalização da resposta imunológica (36, 37).

Medicamentos a base de anticorpos monoclonais como Remdesivir, Faviparir, Rabivirin também foram utilizados em diversos hospitais pelo mundo, mas apresentam alto custos de produção (38, 39, 40, 41). Contudo, sua utilização passa a ser interessante do ponto de vista

econômico uma vez que estudos de custo efetividade demonstraram efeito benéfico em pacientes graves, reduzindo tempo de internação de UTI onde, por exemplo, é um dos setores mais caros da assistência hospitalar (42, 43, 44).

Uma importante terapia alternativa aos medicamentos bastante utilizada no início da pandemia foi a administração do soro convalescente de pacientes que sobreviveram à COVID-19 (45). A abordagem terapêutica do plasma convalescente baseia-se no princípio da terapia de imunização passiva, uma estratégia de curto prazo em que anticorpos do sangue de alguém que se recuperou de uma infecção podem ser administrados para proteger ou tratar outra pessoa como forma de ganhar tempo até que a produção dos anticorpos do receptor atinja níveis adequados para combater a infecção (45, 46).

Embora até o presente momento tenha diversas formas de tratamento para a COVID-19, somente com o desenvolvimento e aplicação das vacinas foram essenciais para conter o avanço da doença mundialmente. Em 2 de julho de 2020, o cenário mundial de vacinas SARS-CoV-2 incluía 158 vacinas candidatas, das quais 135 estavam na fase pré-clínica ou exploratória de seu desenvolvimento como a mRNA-1273 (Moderna), Ad5-nCoV (CanSino Biologicals), INO-4800 (Inovio, Inc.), LV-SMENP-DC, aAPC específico de patógeno (ShinzenGeno-Immune Medical Institute) e ChAdOx1 (Universidade de Oxford) (47). Ainda que existam particulares no desenvolvimento de cada vacina, isto é, podendo utilizar diferentes partes do vírus, e quantidade de doses recomendadas, as vacinas contra COVID-19 são altamente eficazes ou provavelmente altamente eficazes na prevenção da infecção por SARS-CoV-2, COVID-19 sintomático e COVID-19 grave ou crítico (48).

Em relação às vacinas, estão disponíveis no Brasil quatro vacinas para a imunização da população contra a COVID-19, são elas: 1) a vacina adsorvida inativada do laboratório Sinovac em parceria com o Butantan, 2) a vacina com vírus recombinante do laboratório AstraZeneca em parceria com FioCruz, 3) a vacina com RNAm do laboratório Pfizer/Wyeth, 4) e a vacina com vírus recombinante do laboratório Janssen(49). Mais outras duas vacinas também foram utilizadas no início da campanha de imunização: Covaxin do laboratório Bharat Biotech e Sputnik V (50).

O plano de imunização no Brasil seguiu de acordo com grupos de faixa etária e priorizando profissionais de saúde que estavam na linha de frente da COVID-19, idosos, imunocomprometidos, gestantes, pessoas vulneráveis ou institucionalizadas, e por fim população geral (51). Na figura 3 é apresentado o esquema de vacinação utilizado no Brasil.

Figura 3 - Esquema de vacinação contra COVID-19 utilizada no Brasil.

Fonte: Plano Nacional de Operacionalização da vacinação contra a COVID-19. MS/SVS.

EPIDEMIOLOGIA

Ainda é incerto sobre a origem da doença e o paciente 0 que contraiu o vírus (52). Porém, alguns estudos tentam esclarecer por meio de relatos locais de surtos nos momentos que antecedem o primeiro caso notificado em 17 de novembro de 2020 (52, 53, 54). O fato é que desde essa primeira notificação, a doença se espalhou exponencialmente da China para o mundo.

Até o presente momento, a OMS contabilizou mais de 645 milhões de casos e 6,6 milhões de óbitos por COVID-19 no mundo (55). Cerca de 28% do total (~183 milhões) de casos e 43% dos óbitos (~2,8 milhões) foram registrados nas Américas (55) (Tabela 1). Especificamente no Brasil, foram totalizados 36,7 milhões de casos e mais de 696 mil óbitos (56).

Tabela 1 - Número de casos e óbitos novos e acumulados de COVID-19 desde 2020.

Região	**Novos casos nos últimos 7 dias (%)**	**Casos acumulados (%)**	**Novos óbitos nos últimos 7 dias (%)**	**Óbitos acumulados (%)**
Pacífico Ocidental	1.493.218 (45%)	101.362.984 (16%)	2.283 (23%)	287.229 (4%)
Europa	962.317	267.030.169	2.586	2.142.666

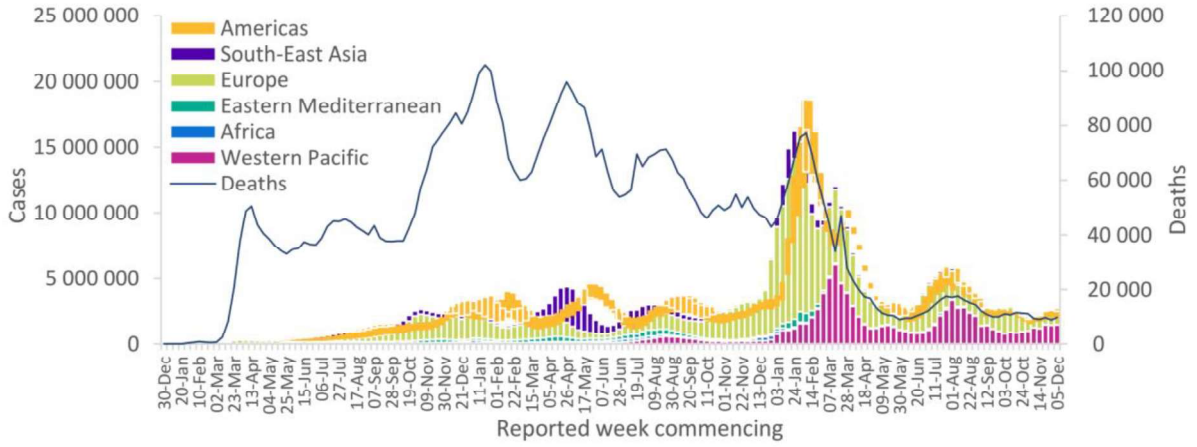
	(29%)	(41%)	(26%)	(32%)
Américas	836.681	183.376.268	4.347	2.877.025
	(25%)	(28%)	(44%)	(43%)
Sudeste da Ásia	24.400	60.703.589	388	802.495
	(1%)	(9%)	(4%)	(12%)
Mediterrâneo	7268	23.206 433	49	348.972
Oriental	(<1%)	(4%)	(1%)	(5%)
África	2826	9.420.467	129	175.063
	(<1%)	(1%)	(1%)	(3%)
Global	3.326.710	645.100.674	9.782	6.633.463
	(100%)	(100%)	(100%)	(100%)

Fonte: World Health Organization: COVID-19 Weekly Epidemiological Update Edition 122 - 14 December 2022 (51).

Na Figura 4 é possível observar repentino aumento de casos periodicamente ao logo do tempo desde o início da pandemia num processo denominado onda de casos. Logo no primeiro ano da pandemia vários países observaram um padrão de duas ondas: uma primeira onda na primavera e uma segunda no final do verão e no outono (57). Esse acontecimento estava relacionado com a alternância das medidas restritivas e de relaxamento no controle da doença (57). Mais tarde em 2021, outros padrões de onda apareçam possivelmente relacionados com o surgimento de variantes do SARS-CoV-2 com maior capacidade de infecção e transmissão (58, 59).

Em 2022 quando a vacina já estava disponível em diversos locais do mundo, observa-se ainda o surgimento de ondas de casos supostamente devido à baixa adesão a vacinação, receio dos efeitos e eficácia, e negacionismos em alguns países (60). Além disso, a falta de acesso e a disponibilidade à vacinas também contribui para o reaparecimento dos casos (61).

Figura 4 – Evolução temporal do número de casos e óbitos de COVID-19 no mundo de acordo com a Organização Mundial da Saúde.



Fonte: World Health Organization: COVID-19 Weekly Epidemiological Update Edition 122 - 14 December 2022 (55).

Atualmente o Brasil ocupa a 5ª posição como o país com maior quantidade de casos de COVID-19, 2º com maior número de óbitos (62) , e o 15º com mais óbitos por milhão de habitantes (63). Em relação à vacinação contra COVID-19, o país está na 25ª posição do rank de imunização, com cerca de 188 milhões de pessoas (~88%) vacinadas com pelo menos a primeira dose da vacina, ao passo que 81,8% foram totalmente vacinadas (63). Ainda que o sistema de saúde do Brasil fosse um dos mais estruturados da América Latina, e até mesmo exemplo mundial a ser seguido, seu desempenho foi afetado por desavenças políticas, falta de recursos pessoais e financeiros (1, 15, 64).

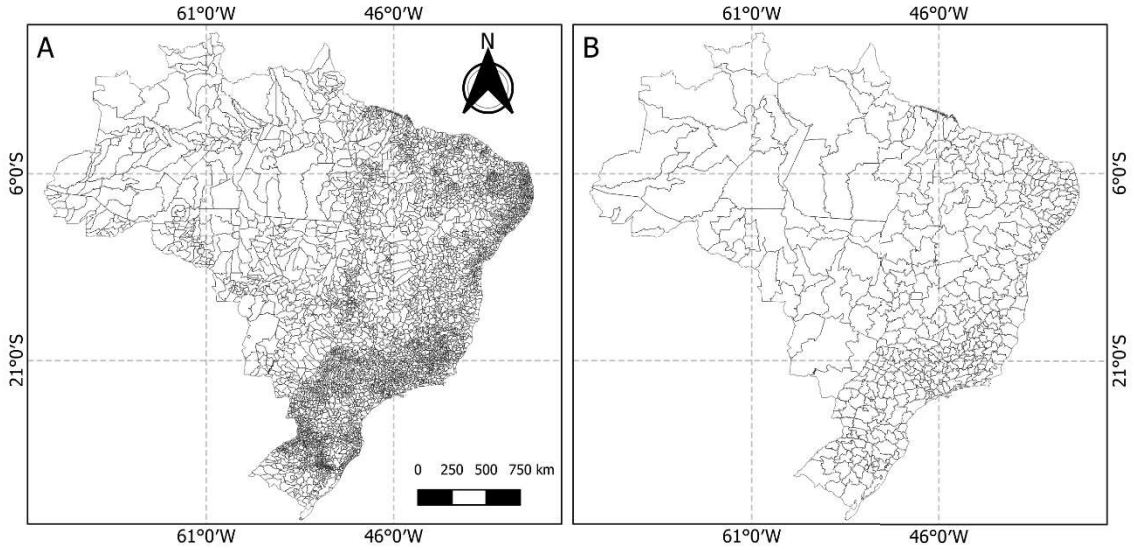
SISTEMA DE SAÚDE DO BRASIL

Logo no início da pandemia, o Brasil estava atento aos eventos da COVID-19 na China ainda em meados de dezembro de 2020, e seguiu as recomendações da OMS para conter o progresso da doença antes mesmo dela ter chego ao país (64). Em 22 de janeiro, o Ministério da Saúde do Brasil, por meio do Decreto nº 188 11, criou o Centro de Operações de Saúde de Emergência contra a COVID-19, em que o objetivo foi responder à emergência SARS-CoV-2 a nível nacional organizando ações coordenadas no Sistema de Saúde, além de o orientar as Secretarias de Saúde, estados, municípios, serviços de saúde públicos e privados, órgãos e empresas sobre planos de resposta que deveriam ser realizados (65).

O Sistema de Saúde do Brasil apresenta características únicas, embora tenha sido criado com base no sistema de saúde do Reino Unido (66). No Brasil é chamado de Sistema Único de Saúde (SUS), e foi construído com base nos princípios da universalidade, integridade e equidade, garantindo o acesso à saúde para toda a população (67). O sistema é descentralizado, o que dá autonomia aos municípios e estados para a execução de suas políticas de saúde com o apoio do governo federal (67). Assim, as comissões intergovernamentais tripartites e bipartites contam com a participação do governo federal, estados e municípios para a tomada de decisões relacionadas às políticas de saúde (67). Além disso, o setor privado pode contribuir com o SUS de forma complementar, beneficiando a população com as instituições de saúde pública e privada (67).

Ainda sobre o processo de descentralização, os municípios criaram um departamento somente para administrar os estabelecimentos de saúde com o objetivo principal de assumir a responsabilidade pelo financiamento dos programas e gestão de serviços de saúde (66). Portanto, cada um dos 5.570 municípios do brasileiros são responsáveis pela promoção de saúde da população em todos os níveis de cuidado a saúde (68). Mas para facilitar o gerenciamento coordenado dos municípios a respeito do cuidado da saúde, atualmente os 5.570 municípios do Brasil estão agrupados em 450 regiões de saúde (Figura 5) (69), onde o conjunto de municípios com interesses sociais, econômicos e de infraestrutura são compartilhados visando maior foco no atendimento das necessidades locais da população (70). Essas regiões de saúde seguem as diretrizes da Agência Nacional de Saúde Vigilância (ANVISA), um órgão federal criada em 1999 com o propósito de controlar a qualidade de medicamentos, produtos de saúde e serviços de saúde (71).

Figura 5 - Limites municiais do Brasil em A, e limites das regiões de saúde em B.



Fonte: Próprio autor.

O SUS foi sobrecarregado durante a pandemia da COVID-19 mediante aos decorrentes cortes financeiros durante anos, e as inconsistências do governo federal sobre o posicionamento frente à pandemia (15). Enquanto por um lado se preocupava com os cuidados da saúde da população, por outro, a economia do país parecia ter sido o foco de maior preocupação (50). Portanto, o resultado do desalinhamento entre os setores do governo federal levou o SUS ao caos (72).

A disponibilidade dos leitos de unidade de terapia intensiva (UTI) para o cuidado de pacientes em estado crítico na fase inicial da pandemia era motivo de muita preocupação quando municípios reportavam esgotamento dos leitos diariamente (73, 74, 75). A exemplo do que ocorreu em outros países, a alta demanda por serviços especializados de saúde característica da COVID- 19 levou a uma situação crítica em que a oferta de leitos de UTI e de ventiladores para atender a demanda de pacientes graves (76, 77). Este esgotamento de recursos, em especial de leitos de UTI, respiradores mecânicos e profissionais habilitados comprometeu a oferta de cuidados tanto a pacientes portadores de COVID-19 quanto a pacientes portadores das demais doenças (1, 78).

Segundo a Associação de Medicina Intensiva Brasileira (AMIB), a proporção de leitos de UTI por 10 mil habitantes em 2020 no Brasil pelo SUS era de 1,4, contra uma proporção de 4,9 no setor privado, ao passo que juntos a proporção era de 2,2, um valor satisfatório de acordo

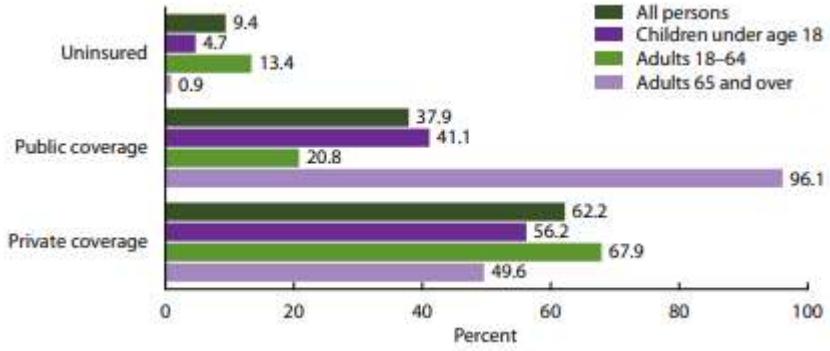
com a AMIB (79). Contudo, trabalhos mostraram que esses leitos estão distribuídos de forma irregular, pois menos de 10% dos municípios brasileiros possuem UTI (80). Em 2016 quando houve um censo para investigar a distribuição dos leitos de UTI no Brasil, verificou-se que havia 41.741 leitos de UTI distribuídos em apenas 521 municípios (81). Destes, cerca de 20.523 pertencem ao SUS, e 21.218 são externos ao SUS. A maioria se concentrava na região sudeste (53,4%), onde o estado do São Paulo representava 24,1% de todos os estabelecimentos com UTI (81), ao passo que a região centro-oeste (8,4%) e norte (5,2%) representavam as regiões com menor quantidade de leitos de UTI do Brasil (81). Ainda em 2016, a região norte apresentava a menor proporção de leitos de UTI por 10 mil habitantes, cerca de 1,23, ao passo que a região sudeste tinha a maior proporção com 2,58. Com relação a mão de obra, no Brasil existiam 11.563 médicos intensivistas, sendo em São Paulo onde se concentrava 28,3% destes profissionais (81).

SISTEMA DE SAÚDE DOS ESTADOS UNIDOS

O Sistema de Saúde dos Estados Unidos da América (EUA), diferentemente do Brasil, não é universal (82), ou seja, não atende os cuidados de saúde universais de que, segundo a OMS, seja "garantir que todas as pessoas tenham acesso aos serviços de saúde necessários (incluindo prevenção, promoção, tratamento, reabilitação e paliação) de qualidade suficiente para serem eficazes, garantindo também que o uso desses serviços não exponha o risco usuário a dificuldade financeira" (83). Nos EUA é utilizado uma abordagem mista entre os serviços públicos e privados, dos quais a população podem obter seguro de saúde de companhias de seguros privados, ou subsidiados pelo governo (82). Entretanto, ainda assim uma parte da população dos EUA não possui seguro de saúde (84).

De acordo com o CDC dos EUA, cerca de 30,4 milhões de americanos (~9,4%) não possuíam seguro de saúde em 2020 (Figura 6) (85). E para piorar, naquele período os EUA foi severamente impactado pela pandemia da COVID-19 o que enalteceu os problemas com saúde pública, não somente pela falta de assistência, mas também falta de recursos hospitalares e profissionais (86, 87).

Figura 6 - Porcentagens de pessoas que estavam sem seguro ou com cobertura pública ou privada no momento da entrevista, por faixa etária: Estados Unidos, janeiro a junho de 2020.



Fonte: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Health Statistics (85).

Outro grande problema do sistema de saúde dos EUA está nos gastos exorbitantes com a saúde, sendo o país que mais gasta com saúde no mundo (88, 89). Além disso, a eficiência do sistema também é questionada se o gasto se justifica pela qualidade. Estudos demonstraram uma baixa eficiência do setor quando avaliado localmente, uma vez que o cuidado também não se dá de forma uniforme pelo país (89).

DETERMINANTES SOCIAIS DA SAÚDE

Durante a história, observou-se que as crises de saúde pública não afetavam as pessoas de maneira uniforme (90). A exemplo disso, a peste negra, que ceifou 2/3 da população global no século XIV, e a gripe espanhola no início do século XX, afetaram principalmente populações mais pobres (91, 92). Portanto, já era de se esperar que os determinantes sociais da saúde seria um dos fatores bem relevantes para a infecção pelo COVID-19 (93).

A Organização Mundial da Saúde define que os determinantes sociais da saúde são as condições e circunstâncias nas quais as pessoas nascem, crescem, vivem, trabalham e envelhecem, e que essas condições são moldadas por forças políticas, sociais e econômicas (Figura 7) (94). Políticas deficientes, planejamento econômico injusto e má governança podem levar a resultados desfavoráveis para a população. As condições sociais, econômicas e políticas de uma sociedade devem garantir que seus cidadãos tenham acesso justo aos recursos (95). A qualidade, quantidade e distribuição desses recursos determinam em grande parte a saúde e o bem-estar de um cidadão. Exemplos desses recursos incluem oportunidades de educação, um ambiente de vida saudável, nutrição, assistência médica e emprego (95).

Figura 7 - Esquema dos determinantes sociais da saúde.

Fonte: Geografia e saúde sem fronteiras (96).

Para demonstrar a importância dos determinantes sociais da saúde, alguns estudos mostraram como a COVID-19 impactou de forma desigual os diferentes grupos populacionais sociais e econômicos (93). Um estudo realizado na Espanha verificou-se que quanto menor o rendimento econômico, maior a incidência de COVID-19 (97). Além disso, a análise de risco relativo, os distritos de renda mais baixa tiveram uma taxa de incidência da doença 2,5 vezes maior do que os distritos de renda mais alta (97). No Reino Unido, indivíduos negros e asiáticos são mais propensos a testarem positivos para COVID-19 quando comparados a brancos (98). Além disso, pessoas vivendo em áreas mais desfavorecidas socioeconomicamente tinham probabilidade maior de testarem positivo para a doença (99). Portanto, é imprescindível que gestores e criadores de políticas públicas tenham em mente a importância desses fatores para elaborarem medidas como forma de prevenção nessas populações.

FERRAMENTAS PARA AVALIAR RECURSOS DE SAÚDE

Atualmente diversos recursos estatísticos são utilizados para auxiliar gestores de saúde a elaborarem estratégias de prevenção à doenças, planos de contingencia e medidas profiláticas para diversas doenças (100). Um exemplo disso, são os Sistemas de Informação Geográfica (SIG), uma ferramenta de análise espacial essencial para a pesquisa, e política de saúde pública (101). O SIG nos permite mapear padrões de doenças, identificar áreas de alto risco e avaliar a eficácia das intervenções, além de pode ser usado para atingir populações em risco, rastrear a propagação de doenças infecciosas e ficar de olho nos riscos à saúde ambiental (102, 103).

Uma metodologia comumente usada é a análise de agrupamento espacial (*clusters*). O CDC define *clusters* como "uma agregação incomum, real ou percebidos, de eventos de saúde que são agrupados no tempo e no espaço" (104), que pode ocorrer em vários tipos de dados baseado na população ou em eventos por exemplo (105). Para cada tipo de dados, numerosas análises de *cluster* existem e variam amplamente em relação a suposições e interpretações (105).

Uma das análises de *cluster* é o Local Indicator of Spatial Association (LISA), que se baseia nos valores padronizados da relação proporcional entre a soma das estatísticas locais e uma global (106). O resultado dessa padronização permite uma classificação dos locais significativos como *clusters* Alto-Alto, quando os valores de um município e seus vizinhos adjacentes são altos em relação ao valor global, e Baixo-Baixo, quando contrário, e os *outliers* (valores discrepantes dos valores limites padrão) Alto-Baixo e Baixo-Alto. Uma alternativa a essa análise é o Getis-Ord-Gi*, que classifica os *clusters* em hotspots (valores positivos ou maiores do que a média do valor padronizado de Z) ou coldspots (valores negativos ou abaixo da média do valor padronizado de Z) (107), em que o score de Z é um teste que avalia o desvio padrão do valor das observações. A soma local para um município e seus vizinhos é comparada proporcionalmente à soma de todos os recursos, e a partir disso, quando a soma local é muito diferente da soma local esperada, e quando essa diferença é muito grande para ser o resultado do acaso, resulta um escore Z estatisticamente significativo (107). Outra análise de *cluster* é o *multivariate clustering*. Trata-se de um dos recursos do aprendizado de máquina do algoritmo *K-means* utilizado para identificar agrupamentos baseado nas características em comum ao utilizar variáveis sociodemográficas.

A acessibilidade aos recursos de saúde é uma importante variável para verificar o desempenho do sistema de saúde local de uma região (1, 108, 109). O método de área de captação flutuante de duas etapas (*2-step floating catchment área:* 2SFCA) está entre os

métodos mais comumente usados para medir a acessibilidade espacial, especialmente de saúde (108). Neste método, leva em consideração a duração máxima aceitável para tempo ou distância Euclidiana de viagem, e os pontos de oferta ou demanda apenas dentro desse limiar, e a análise é realizada em dois passos: o primeiro passo é procurar os locais da população e dos pontos de oferta dentro do limiar, e calcular a relação oferta-demanda de cada instalação. Na segunda etapa, para cada estabelecimento, determina-se o número de instalações dentro do limite e somam-se as razões de oferta e demanda de todas as instalações para obter a acessibilidade espacial do assentamento. O resultado é uma taxa de acessibilidade, por exemplo, quantidade de leitos hospitalares por 10 mil habitantes dentro de uma determinada distância e tempo (1, 108).

Contudo, muitos fatores podem influenciar o acesso aos serviços de saúde, como a disponibilidade de postos de saúde na área (oferta), o tamanho da população da área (demanda), e as barreiras geográficas entre oferta e demanda (110). Portanto, outra ferramenta importante para melhorias do sistema de saúde a respeito de cobertura assistencial é a análise de localização-alocação *(location-allocation)*, a qual permite rearranjar estabelecimentos já existentes a fim de direcionar recursos novos para melhorar a cobertura da população (111). Assim, o modelo de localização-alocação é um problema de tomada de decisão estratégica que visa identificar as localizações ótimas para instalações que envolvem simultaneamente identificar um conjunto de localizações de instalações e atribuir conjuntos de demandas espacialmente distribuídas a essas instalações com o objetivo de melhorar a distribuição existente. Para essa análise é preciso: 1º) a demanda: podendo ser a população, 2º) oferta: locais de estabelecimento já existentes e os estabelecimentos candidatos; 3º o tipo de algoritmo para encontrar os melhores estabelecimentos (110). Um dos algoritmos utilizados foi proposto por Holmes et al. (1972) chamado de *Maximize attendance,* que busca maximizar o número de pontos de demanda que uma instalação pode atender em um determinado limite de impedância. Este problema de maximização assume que a interação entre as localizações dos estabelecimentos e as localizações dos pontos de demanda diminuem com o aumento da distância, ou seja, as localizações ótimas dos estabelecimentos estarão localizados bem próximas ao maior número de pontos de demanda (112).

OBJETIVOS

GERAL

Avaliar a disponibilidade de recursos do sistema de saúde para dar suporte à população brasileira durante a COVID-19. Além disso, verificar a relação dos determinantes sociais de saúde com os casos de COVID-19 numa cidade de médio porte nos Estados Unidos da América.

ESPECÍFICOS

- Avaliar a disponibilidade: leitos hospitalares normais, leitos de UTI, ventiladores hospitalares, e profissionais da saúde, bem como sua distribuição pelo território brasileiro a fim de determinar se atendem as recomendações da ANVISA;
- Aplicar uma análise espacial utilizando 2SFCA para verificar o acesso da população aos leitos hospitalares no Brasil;
- Verificar a distribuição de vacinas para a primeira dose contra COVID-19 num estado do sul do Brasil, e, posteriormente, aplicar uma análise de *location-allocation* para determinar os possíveis locais para se destinarem as vacinas a fim de cobrir uma maior população;
- Determinar a distribuição de casos de COVID-19 de uma população atendida por um hospital particular da cidade de Durham, Carolina do Norte, EUA. Em seguida, coletar diversos indicadores socioeconômicos do censo dos EUA, para então verificar se esses indicadores se correlacionam com os casos da doença por meio da análise *multivariate clustering*.

REFERÊNCIAS

1. Silva LL, de Carvalho Dutra A, de Andrade L, Iora PH, Rodrigues Ramajo GL, Peres Gualda IA, et al. Emergency Care Gap in Brazil: Geographical Accessibility as a Proxy of Response Capacity to Tackle COVID-19. Front Public Health. 2021;9:740284.
2. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020;382(8):727-33.
3. Siddiqui S, Alhamdi HWS, Alghamdi HA. Recent Chronology of COVID-19 Pandemic. Front Public Health. 2022;10:778037.
4. Hu B, Guo H, Zhou P, Shi ZL. Characteristics of SARS-CoV-2 and COVID-19. Nat Rev Microbiol. 2021;19(3):141-54.
5. Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y, et al. Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus-Infected Pneumonia. N Engl J Med. 2020;382(13):1199-207.
6. WHO (World Health Organization). COVID 19 Public Health Emergency of International Concern (PHEIC). Global research and innovation forum: towards a research roadmap. 2020.
7. WHO (World Health Organization). Coronavirus disease (COVID-19): situation reports. 2020.
8. Li Z, Jones C, Ejigu GS, George N, Geller AL, Chang GC, et al. Countries with delayed COVID-19 introduction - characteristics, drivers, gaps, and opportunities. Global Health. 2021;17(1):28.
9. Zeiser FA, Donida B, da Costa CA, Ramos GO, Scherer JN, Barcellos NT, et al. First and second COVID-19 waves in Brazil: A cross-sectional study of patients' characteristics related to hospitalization and in-hospital mortality. Lancet Reg Health Am. 2022;6:100107.
10. Burki T. COVID-19 in Latin America. Lancet Infect Dis. 2020;20(5):547-8.
11. The L. The unfolding migrant crisis in Latin America. Lancet. 2019;394(10213):1966.
12. Hotez PJ, Bottazzi ME, Franco-Paredes C, Ault SK, Periago MR. The neglected tropical diseases of Latin America and the Caribbean: a review of disease burden and distribution and a roadmap for control and elimination. PLoS Negl Trop Dis. 2008;2(9):e300.
13. Rodriguez-Morales AJ, Gallego V, Escalera-Antezana JP, Méndez CA, Zambrano LI, Franco-Paredes C, et al. COVID-19 in Latin America: The implications of the first confirmed case in Brazil. Travel Med Infect Dis. 2020;35:101613.
14. Cimerman S, Chebabo A, Cunha CAD, Rodriguez-Morales AJ. Deep impact of COVID-19 in the healthcare of Latin America: the case of Brazil. Braz J Infect Dis. 2020.
15. Sott MK, Bender MS, da Silva Baum K. Covid-19 Outbreak in Brazil: Health, Social, Political, and Economic Implications. Int J Health Serv. 2022;52(4):442-54.
16. Naqvi AAT, Fatima K, Mohammad T, Fatima U, Singh IK, Singh A, et al. Insights into SARS-CoV-2 genome, structure, evolution, pathogenesis and therapies: Structural genomics approach. Biochim Biophys Acta Mol Basis Dis. 2020;1866(10):165878.
17. Cui J, Li F, Shi ZL. Origin and evolution of pathogenic coronaviruses. Nat Rev Microbiol. 2019;17(3):181-92.
18. Wang MY, Zhao R, Gao LJ, Gao XF, Wang DP, Cao JM. SARS-CoV-2: Structure, Biology, and Structure-Based Therapeutics Development. Front Cell Infect Microbiol. 2020;10:587269.
19. Zhang Q, Xiang R, Huo S, Zhou Y, Jiang S, Wang Q, et al. Molecular mechanism of interaction between SARS-CoV-2 and host cells and interventional therapy. Signal Transduct Target Ther. 2021;6(1):233.

20. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020;579(7798):270-3.
21. Cheng VC, Lau SK, Woo PC, Yuen KY. Severe acute respiratory syndrome coronavirus as an agent of emerging and reemerging infection. Clin Microbiol Rev. 2007;20(4):660-94.
22. Takeda M. Proteolytic activation of SARS-CoV-2 spike protein. Microbiol Immunol. 2022;66(1):15-23.
23. Santos IA, Grosche VR, Bergamini FRG, Sabino-Silva R, Jardim ACG. Antivirals Against Coronaviruses: Candidate Drugs for SARS-CoV-2 Treatment? Front Microbiol. 2020;11:1818.
24. Kumar A, Narayan RK, Prasoon P, Kumari C, Kaur G, Kumar S, et al. COVID-19 Mechanisms in the Human Body-What We Know So Far. Front Immunol. 2021;12:693938.
25. Sudre CH, Murray B, Varsavsky T, Graham MS, Penfold RS, Bowyer RC, et al. Attributes and predictors of long COVID. Nat Med. 2021;27(4):626-31.
26. Kumar A, Prasoon P, Sekhawat PS, Pareek V, Faiq MA, Kumari C, et al. Pathogenesis guided therapeutic management of COVID-19: an immunological perspective. Int Rev Immunol. 2021;40(1-2):54-71.
27. Esakandari H, Nabi-Afjadi M, Fakkari-Afjadi J, Farahmandian N, Miresmaeili SM, Bahreini E. A comprehensive review of COVID-19 characteristics. Biol Proced Online. 2020;22:19.
28. Baj J, Karakuła-Juchnowicz H, Teresiński G, Buszewicz G, Ciesielka M, Sitarz R, et al. COVID-19: Specific and Non-Specific Clinical Manifestations and Symptoms: The Current State of Knowledge. J Clin Med. 2020;9(6).
29. Hernandez Acosta RA, Esquer Garrigos Z, Marcelin JR, Vijayvargiya P. COVID-19 Pathogenesis and Clinical Manifestations. Infect Dis Clin North Am. 2022;36(2):231-49.
30. Hindson J. COVID-19: faecal-oral transmission? Nat Rev Gastroenterol Hepatol. 2020;17(5):259.
31. Li H, Wang Y, Ji M, Pei F, Zhao Q, Zhou Y, et al. Transmission Routes Analysis of SARS-CoV-2: A Systematic Review and Case Report. Front Cell Dev Biol. 2020;8:618.
32. Ministério da S. Guia de vigilância epidemiológica emergência de saúde pública de importância nacional pela doença pelo coronavírus 2019. Ministério da Saúde Brasília; 2020.
33. Lapi F, Marconi E, Grattagliano I, Rossi A, Fornasari D, Magni A, et al. To clarify the safety profile of paracetamol for home-care patients with COVID-19: a real-world cohort study, with nested case-control analysis, in primary care. Intern Emerg Med. 2022;17(8):2237-44.
34. Perico N, Cortinovis M, Suter F, Remuzzi G. Home as the new frontier for the treatment of COVID-19: the case for anti-inflammatory agents. Lancet Infect Dis. 2023;23(1):e22-e33.
35. Niknam Z, Jafari A, Golchin A, Danesh Pouya F, Nemati M, Rezaei-Tavirani M, et al. Potential therapeutic options for COVID-19: an update on current evidence. Eur J Med Res. 2022;27(1):6.
36. Sallard E, Lescure FX, Yazdanpanah Y, Mentre F, Peiffer-Smadja N. Type 1 interferons as a potential treatment against COVID-19. Antiviral Res. 2020;178:104791.
37. Kopecky-Bromberg SA, Martínez-Sobrido L, Frieman M, Baric RA, Palese P. Severe acute respiratory syndrome coronavirus open reading frame (ORF) 3b, ORF 6, and nucleocapsid proteins function as interferon antagonists. J Virol. 2007;81(2):548-57.
38. Beigel JH, Tomashek KM, Dodd LE, Mehta AK, Zingman BS, Kalil AC, et al. Remdesivir for the Treatment of Covid-19 - Final Report. N Engl J Med. 2020;383(19):1813-26.

39. Du YX, Chen XP. Favipiravir: Pharmacokinetics and Concerns About Clinical Trials for 2019-nCoV Infection. Clin Pharmacol Ther. 2020;108(2):242-7.
40. Elfiky AA. Ribavirin, Remdesivir, Sofosbuvir, Galidesivir, and Tenofovir against SARS-CoV-2 RNA dependent RNA polymerase (RdRp): A molecular docking study. Life Sci. 2020;253:117592.
41. Flaxman AD, Issema R, Barnabas RV, Ross JM. Estimated Health Outcomes and Costs of COVID-19 Prophylaxis With Monoclonal Antibodies Among Unvaccinated Household Contacts in the US. JAMA Netw Open. 2022;5(4):e228632.
42. Miguez-Rey E, Choi D, Kim S, Yoon S, Săndulescu O. Monoclonal antibody therapies in the management of SARS-CoV-2 infection. Expert Opin Investig Drugs. 2022;31(1):41-58.
43. Jovanoski N, Kuznik A, Becker U, Hussein M, Briggs A. Cost-effectiveness of casirivimab/imdevimab in patients with COVID-19 in the ambulatory setting. J Manag Care Spec Pharm. 2022;28(5):555-65.
44. Carta A, Conversano C. Cost utility analysis of Remdesivir and Dexamethasone treatment for hospitalised COVID-19 patients - a hypothetical study. BMC Health Serv Res. 2021;21(1):986.
45. Montelongo-Jauregui D, Vila T, Sultan AS, Jabra-Rizk MA. Convalescent serum therapy for COVID-19: A 19th century remedy for a 21st century disease. PLoS Pathog. 2020;16(8):e1008735.
46. Casadevall A, Pirofski LA. The convalescent sera option for containing COVID-19. J Clin Invest. 2020;130(4):1545-8.
47. Kaur SP, Gupta V. COVID-19 Vaccine: A comprehensive status report. Virus Res. 2020;288:198114.
48. Graña C, Ghosn L, Evrenoglou T, Jarde A, Minozzi S, Bergman H, et al. Efficacy and safety of COVID-19 vaccines. Cochrane Database Syst Rev. 2022;12(12):Cd015477.
49. Brasil. Ministério da, Saúde. Plano nacional de operacionalização da vacinação contra a COVID-19. Ministério da Saúde Brasília (DF); 2022.
50. Bernardeau-Serra L, Nguyen-Huynh A, Sponagel L, Sernizon Guimarães N, Teixeira de Aguiar RA, Soriano Marcolino M. The COVID-19 Vaccination Strategy in Brazil-A Case Study. Epidemiologia (Basel). 2021;2(3):338-59.
51. Brasil. Ministério da Saúde Secretaria de Vigilância em Saúde. NOTA INFORMATIVA Nº 6/2021-CGPNI/DEIDT/SVS/MS 2021 [Internet]. [cited 2023 Jan 19]. Available from: https://sbim.org.br/images/files/notas-tecnicas/nota-informativa-6-2021-cgpni-deidt-svs-ms.pdf.
52. Lu D. The hunt to find the coronavirus pandemic's patient zero. New Sci. 2020;245(3276):9.
53. Feng M, Ling Q, Xiong J, Manyande A, Xu W, Xiang B. Occupational Characteristics and Management Measures of Sporadic COVID-19 Outbreaks From June 2020 to January 2021 in China: The Importance of Tracking Down "Patient Zero". Front Public Health. 2021;9:670669.
54. Tichopád A, Pecen L, Sedlák V. Could the new coronavirus have infected humans prior November 2019? PLoS One. 2021;16(8):e0248255.
55. World Health O. Weekly epidemiological update on COVID-19–28 December 2021. 2022.
56. Dong E, Ratcliff J, Goyea TD, Katz A, Lau R, Ng TK, et al. The Johns Hopkins University Center for Systems Science and Engineering COVID-19 Dashboard: data collection process, challenges faced, and lessons learned. The Lancet Infectious Diseases. 2022.

57. Iftimie S, López-Azcona AF, Vallverdú I, Hernández-Flix S, de Febrer G, Parra S, et al. First and second waves of coronavirus disease-19: A comparative study in hospitalized patients in Reus, Spain. PLoS One. 2021;16(3):e0248029.
58. Islam S, Islam T, Islam MR. New Coronavirus Variants are Creating More Challenges to Global Healthcare System: A Brief Report on the Current Knowledge. Clin Pathol. 2022;15:2632010x221075584.
59. Samieefar N, Rashedi R, Akhlaghdoust M, Mashhadi M, Darzi P, Rezaei N. Delta Variant: The New Challenge of COVID-19 Pandemic, an Overview of Epidemiological, Clinical, and Immune Characteristics. Acta Biomed. 2022;93(1):e2022179.
60. Sallam M. COVID-19 Vaccine Hesitancy Worldwide: A Concise Systematic Review of Vaccine Acceptance Rates. Vaccines (Basel). 2021;9(2).
61. Fan G, Song H, Yip S, Zhang T, He D. Impact of low vaccine coverage on the resurgence of COVID-19 in Central and Eastern Europe. One Health. 2022;14:100402.
62. WHO (World Health Organization). Dashboard [Internet]. [cited 2023 Jan 19]. Available from: https://covid19.who.int.
63. Ritchie H, Mathieu E, Rodés-Guirao L, Appel C, Giattino C, Ortiz-Ospina E, et al. Coronavirus pandemic (COVID-19). Published online at OurWorldInData. org. Retrieved from:[Online Resource]. https://ourworldindata. org/coronavirus; 2020.
64. Croda J, Oliveira WK, Frutuoso RL, Mandetta LH, Baia-da-Silva DC, Brito-Sousa JD, et al. COVID-19 in Brazil: advantages of a socialized unified health system and preparation to contain cases. Rev Soc Bras Med Trop. 2020;53:e20200167.
65. Brasil, Ministério da S. Plano de contingência nacional para infecção humana pelo novo coronavírus COVID-19. MS Brasília; 2020.
66. Castro MC, Massuda A, Almeida G, Menezes-Filho NA, Andrade MV, de Souza Noronha KVM, et al. Brazil's unified health system: the first 30 years and prospects for the future. Lancet. 2019;394(10195):345-56.
67. Brasil CC, Brasil. Decreto nº 7.508, de 28 de junho de 2011. Regulamenta a Lei no 8.080, de 19 de setembro de 1990, para dispor sobre a organização do Sistema Único de Saúde-SUS, o planejamento da saúde, a assistência à saúde ea articulação interfederativa, e dá outras providências. Diário Oficial da União. 2011;1.
68. Giovanella L, Escorel S, Lobato LdVC, de Carvalho Noronha J, de Carvalho AI. Políticas e sistema de saúde no Brasil: SciELO-Editora FIOCRUZ; 2012.
69. Pereira CCA, Soares FRG, Machado CJ, Frio GS, Alves LC, Herkrath FJ, et al. Development of an Index to Assess COVID-19 Hospital Care Installed Capacity in the 450 Brazilian Health Regions. Disaster Med Public Health Prep. 2022:1-8.
70. Viana ALdÁ, Ferreira MP, Cutrim MA, Fusaro ER, Souza MRd, Mourão L, et al. The regionalization process in Brazil: influence on policy, structure and organization dimensions. Revista Brasileira de Saúde Materno Infantil. 2017;17:S27-S43.
71. Brasil. Decreto n. 3.029, de 16 de abril de 1999: Presidente da República aprova o Regulamento da Agência Nacional de Vigilância Sanitária. Diário Oficial da União. 1999(73).
72. Szylovec A, Umbelino-Walker I, Cain BN, Ng HT, Flahault A, Rozanova L. Brazil's Actions and Reactions in the Fight Against COVID-19 from January to March 2020. Int J Environ Res Public Health. 2021;18(2).
73. Lemos DRQ, D'Angelo SM, Farias L, Almeida MM, Gomes RG, Pinto GP, et al. Health system collapse 45 days after the detection of COVID-19 in Ceará, Northeast Brazil: a preliminary analysis. Rev Soc Bras Med Trop. 2020;53:e20200354.
74. Mais de 4.000 pessoas com COVID-19 morreram à espera por um leito de UTI em seis Estados brasileiros. El País [Internet]. [cited 2023 Jan 22]. Available from: https://brasil.elpais.com/brasil/2020-08-26/mais-de-4000-pessoas-com-covid-19-morreram-a-espera-por-um-leito-de-uti-em-seis-estados-brasileiros.html.

75. Nove em cada 10 cidades do país não têm leito de UTI e "exportam" pacientes. UOL [Internet]. [cited 2023 Jan 22]. Available from: https://noticias.uol.com.br/saude/ultimas-noticias/redacao/2020/03/19/nove-em-cada-10-cidades-do-pais-nao-tem-leito-de-uti-e-exportam-pacientes.htm.
76. Grasselli G, Zangrillo A, Zanella A, Antonelli M, Cabrini L, Castelli A, et al. Baseline Characteristics and Outcomes of 1591 Patients Infected With SARS-CoV-2 Admitted to ICUs of the Lombardy Region, Italy. Jama. 2020.
77. Carenzo L, Costantini E, Greco M, Barra FL, Rendiniello V, Mainetti M, et al. Hospital surge capacity in a tertiary emergency referral centre during the COVID-19 outbreak in Italy. Anaesthesia. 2020.
78. Kretzer L, Berbigier E, Lisboa R, Grumann AC, Andrade J. Protocolo AMIB de alocação de recursos em esgotamento durante a pandemia por covid-19. Associação de Medicina Intensiva Brasileira[Versão eletrônica] Obtido em. 2020;28.
79. AMIB apresenta dados atualizados sobre leitos de UTI no Brasil. Associação de Medicina Intensiva Brasileira [internet]. [cited 2023 Jan 22]. Available from: http://www.epsjv.fiocruz.br/sites/default/files/files/dados_uti_amib%281%29.pdf.
80. Batista Filho LAC, Randhawa VK, Maciel AT, Coimbra MR. We need to talk about critical care in Brazil. Clinics (Sao Paulo). 2022;77:100096.
81. Associação de Medicina Brasileira (AMIB). Panorama da Medicina Intensiva no Brasil em Dezembro 2016. Censo AMIB 2016 [Internet]. São Paulo: AMIB; 2016. [cited 2023 Jan 22]. Available from: http://www.amib.org.br/fileadmin/user_upload/amib/2018/marco/19/Analise_de_Dados_UTI_Final.pdf.
82. Zieff G, Kerr ZY, Moore JB, Stoner L. Universal Healthcare in the United States of America: A Healthy Debate. Medicina (Kaunas). 2020;56(11).
83. World Health O. Universal health coverage. 2013.
84. Davis K. Uninsured in America: problems and possible solutions. Bmj. 2007;334(7589):346-8.
85. Cohen RA, Terlizzi EP, Cha AE, Martinez ME. Health insurance coverage: early release of estimates from the National Health Interview Survey, January–June 2020. 2021.
86. Janke AT, Mei H, Rothenberg C, Becher RD, Lin Z, Venkatesh AK. Analysis of Hospital Resource Availability and COVID-19 Mortality Across the United States. J Hosp Med. 2021;16(4):211-4.
87. Wu H, Soe MM, Konnor R, Dantes R, Haass K, Dudeck MA, et al. Hospital capacities and shortages of healthcare resources among US hospitals during the coronavirus disease 2019 (COVID-19) pandemic, National Healthcare Safety Network (NHSN), March 27-July 14, 2020. Infect Control Hosp Epidemiol. 2022;43(10):1473-6.
88. The most expensive health care system in the world. Harvard T.H. Chan School of Public Health [internet]. [cited 2023 Jan 23]. Available from: https://www.hsph.harvard.edu/news/hsph-in-the-news/the-most-expensive-health-care-system-in-the-world/.
89. Garber AM, Skinner J. Is American health care uniquely inefficient? J Econ Perspect. 2008;22(4):27-50.
90. Ahmed F, Ahmed N, Pissarides C, Stiglitz J. Why inequality could spread COVID-19. Lancet Public Health. 2020;5(5):e240.
91. Duncan CJ, Scott S. What caused the Black Death? Postgrad Med J. 2005;81(955):315-20.
92. Mamelund SE. 1918 pandemic morbidity: The first wave hits the poor, the second wave hits the rich. Influenza Other Respir Viruses. 2018;12(3):307-13.

93. Morante-García W, Zapata-Boluda RM, García-González J, Campuzano-Cuadrado P, Calvillo C, Alarcón-Rodríguez R. Influence of Social Determinants of Health on COVID-19 Infection in Socially Vulnerable Groups. Int J Environ Res Public Health. 2022;19(3).
94. Commission on Social Determinants of H. Closing the gap in a generation: health equity through action on the social determinants of health: final report of the commission on social determinants of health: World Health Organization; 2008.
95. Islam MM. Social Determinants of Health and Related Inequalities: Confusion and Implications. Front Public Health. 2019;7:11.
96. Guimarães RB, Pickenhayn JA, Lima SdC. Geografia e saúde sem fronteiras. Geografia e saúde sem fronteiras2015. p. 160-.
97. Baena-Díez JM, Barroso M, Cordeiro-Coelho SI, Díaz JL, Grau M. Impact of COVID-19 outbreak by income: hitting hardest the most deprived. J Public Health (Oxf). 2020;42(4):698-703.
98. Hastie CE, Mackay DF, Ho F, Celis-Morales CA, Katikireddi SV, Niedzwiedz CL, et al. Vitamin D concentrations and COVID-19 infection in UK Biobank. Diabetes Metab Syndr. 2020;14(4):561-5.
99. de Lusignan S, Dorward J, Correa A, Jones N, Akinyemi O, Amirthalingam G, et al. Risk factors for SARS-CoV-2 among patients in the Oxford Royal College of General Practitioners Research and Surveillance Centre primary care network: a cross-sectional study. Lancet Infect Dis. 2020;20(9):1034-42.
100. Panovska-Griffiths J, Kerr CC, Waites W, Stuart RM. Mathematical modeling as a tool for policy decision making: Applications to the COVID-19 pandemic. 44: Copyright © 2021 Elsevier B.V. All rights reserved.; 2021. p. 291-326.
101. Elwood S. Geographic information science: Visualization, visual methods, and the geoweb. Progress in Human Geography. 2011;35(3):401-8.
102. Ahasan R, Hossain MM. Leveraging GIS and spatial analysis for informed decision-making in COVID-19 pandemic. Health Policy Technol. 2020.
103. Higgs G, Langford M, Jarvis P, Page N, Richards J, Fry R. Using Geographic Information Systems to investigate variations in accessibility to 'extended hours' primary healthcare provision. Health Soc Care Community. 2019;27(4):1074-84.
104. Cdc AZ. Guidelines for investigating clusters of health events. MMWR Recomm Rep. 1990;39:1-23.
105. Fritz CE, Schuurman N, Robertson C, Lear S. A scoping review of spatial cluster analysis techniques for point-event data. Geospat Health. 2013;7(2):183-98.
106. Anselin L. Local Indicators of Spatial Association—LISA. Geographical Analysis. 1995;27(2):93-115.
107. Anselin L. Local spatial autocorrelation. Other Local Spatial Autocorrelation Statistics. 2020.
108. Liu S, Wang Y, Zhou D, Kang Y. Two-Step Floating Catchment Area Model-Based Evaluation of Community Care Facilities' Spatial Accessibility in Xi'an, China. Int J Environ Res Public Health. 2020;17(14).
109. Ngui AN, Apparicio P. Optimizing the two-step floating catchment area method for measuring spatial accessibility to medical clinics in Montreal. BMC Health Serv Res. 2011;11:166.
110. Polo G, Acosta CM, Ferreira F, Dias RA. Location-allocation and accessibility models for improving the spatial planning of public health services. PLoS One. 2015;10(3):e0119190.
111. Rahman S-u, Smith DK. Use of location-allocation models in health service development planning in developing nations. Elsevier; 2000.
112. Holmes J, Williams FB, Brown LA. Facility location under a maximum travel restriction: An example using day care facilities. Geographical Analysis. 1972;4(3):258-66.

CAPÍTULO II

Artigo 1: “Emergency Care Gap in Brazil: Geographical Accessibility as a Proxy of Response Capacity to Tackle COVID-19”

frontiers in Public Health

ORIGINAL RESEARCH
published: 16 November 2021
doi: 10.3389/fpubh.2021.740284

Emergency Care Gap in Brazil: Geographical Accessibility as a Proxy of Response Capacity to Tackle COVID-19

Lincoln Luís Silva[1], *Amanda de Carvalho Dutra*[2], *Luciano de Andrade*[2,3], *Pedro Henrique Iora*[3], *Guilherme Luiz Rodrigues Ramajo*[3], *Iago Amado Peres Gualda*[3], *João Felipe Hermann Costa Scheidt*[3], *Pedro Vasconcelos Maia do Amaral*[4], *Thiago Augusto Hernandes Rocha*[5], *Catherine Ann Staton*[5], *João Ricardo Nickenig Vissoci*[5†] *and Rosilene Fressatti Cardoso*[1*†]

[1] Graduate Program in Biosciences and Physiopathology, State University of Maringá, Paraná, Brazil, [2] Graduate Program in Health Sciences, State University of Maringá, Paraná, Brazil, [3] Department of Medicine, State University of Maringá, Paraná, Brazil, [4] Faculty of Economic Sciences, Federal University of Minas Gerais, Belo Horizonte, Brazil, [5] Global Emergency Medicine Innovation and Implementation (GEMINI), Division of Emergency Medicine, Duke Global Health Institute, Durham, NC, United States

Background: The new coronavirus disease (COVID-19) has claimed thousands of lives worldwide and disrupted the health system in many countries. As the national emergency care capacity is a crucial part of the COVID-19 response, we evaluated the Brazilian Health Care System response preparedness against the COVID-19 pandemic.

Methods: A retrospective and ecological study was performed with data retrieved from the Brazilian Information Technology Department of the Public Health Care System. The numbers of intensive care (ICU) and hospital beds, general or intensivist physicians, nurses, nursing technicians, physiotherapists, and ventilators from each health region were extracted. Beds per health professionals and ventilators per population rates were assessed. A health service accessibility index was created using a two-step floating catchment area (2SFCA). A spatial analysis using Getis-Ord Gi* was performed to identify areas lacking access to high-complexity centers (HCC).

Results: As of February 2020, Brazil had 35,682 ICU beds, 426,388 hospital beds, and 65,411 ventilators. In addition, 17,240 new ICU beds were created in June 2020. The South and Southeast regions have the highest rates of professionals and infrastructure to attend patients with COVID-19 compared with the northern region. The north region has the lowest accessibility to ICUs.

Conclusions: The Brazilian Health Care System is unevenly distributed across the country. The inequitable distribution of health facilities, equipment, and human resources

OPEN ACCESS

Edited by:
Connie J. Evashwick, George Washington University, United States

Reviewed by:
Carolina Ruiz, Pontificia Universidad Católica de Chile, Chile
Ana Maria Malik, Escola de Administração da Fundação Getulio Vargas (FGV-EAESP), Brazil

***Correspondence:**
Rosilene Fressatti Cardoso
rfcardoso@uem.br

[†] These authors have contributed equally to this work and share last authorship

Specialty section:
This article was submitted to Public Health Policy, a section of the journal Frontiers in Public Health

Received: *12 July 2021*
Accepted: *07 October 2021*
Published: *16 November 2021*

Citation:
Silva LL, Carvalho Dutra Ad, Andrade Ld, Iora PH, Rodrigues Ramajo GL, Peres Gualda IA, Costa Scheidt JFH, Vasconcelos Maia do Amaral P, Hernandes Rocha TA, Staton CA, Nickenig Vissoci JR and Fressatti Cardoso R (2021) Emergency Care Gap in Brazil: Geographical Accessibility as a Proxy of Response Capacity to Tackle COVID-19. Front. Public Health 9:740284. doi: 10.3389/fpubh.2021.740284

led to inadequate preparedness to manage the COVID-19 pandemic. In addition, the ineffectiveness of public measures of the municipal and federal administrations aggravated the pandemic in Brazil.

Keywords: COVID-19, epidemiology, spatial analysis, public health, health services accessibility

INTRODUCTION

In December 2019, the novel severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) was identified as the causative agent of coronavirus 2019 (COVID-19) (1). The WHO declared COVID-19 a pandemic disease by the end of January 2020 (1). As of July 6, 2021, COVID-19 infected more than 183 million people and resulted in almost 4 million deaths globally (2). However, Brazil, where the virus reached much later than other countries, is one of the most affected countries with 19.1 million confirmed cases and 533,000 deaths reported so far (2). This case burden put the Brazilian health service at risk of total collapse (3).

To provide necessary health system resources to adequately overcome COVID-19, a strong coordinated emergency response with adequate facilities, personnel, and equipment is required (4). Resource constraints, including a lack of intensive care, inequitable distribution of hospital beds, and inadequate numbers of ventilators, compromise the capacity of the health system to care for patients with COVID-19 (5). In addition, the Brazilian government has faced several challenges while seeking the funds for the public Unified Health System (SUS) over the last decade (6). The lack of funds to support the public health system has led to a deterioration of health capacity among smaller municipalities and exacerbating inequalities (6).

The Unified Health System (Sistema Único de Saúde, SUS) of Brazil is the Brazil Health System. Brazil is the only country with a population of more than 200 million people to have a universal health care system, where almost 75% of the population rely on the service (7). SUS is maintained by public power, with supplementary participation of private initiatives, which guarantees free health care for all citizens, which is based primarily on equity, universality, and integrality (7, 8). However, as Brazil is a country of continental dimensions (8), geographic distribution of hospitals and physicians does not necessarily reach all Brazilians, turning healthcare accessibility into a severe problem. Many studies have recently used the Geographic Information System (GIS) to verify the accessibility to healthcare or to identify poorly served areas (9–11). The two-step floating catchment area (2SFCA) is, by far, the most used GIS technique to verify the accessibility to care, calculating the ratio of suppliers to the population within a service area centered at the location of a supplier (12). Such a method provides useful information on resources management by addressing the necessary support to the most deprived location of the country.

Globally, healthcare systems have collapsed due to COVID-19 (13). Hospital administrators, governments, policy-makers, and researchers must prepare for an immense surge of patients overloading the healthcare system. Thus, to verify the Brazilian healthcare preparedness in pandemic times, this study aims to analyze the geospatial distribution of healthcare resources (intensive care units and hospital beds, ventilators, and hospitals) and the access to establishments with intensive care units (ICU).

MATERIALS AND METHODS

Study Design

This is an ecological, observational, and cross-sectional study based on secondary data. The aim was to evaluate the Brazilian health system infrastructure based on the amount of health resources (ICU and hospital beds, health professionals, and the accessibility to ICU beds) to verify the Brazilian preparedness for the COVID-19 pandemic.

The Brazilian Health System

The SUS is the Brazilian public health system created by the Brazilian Constitution of 1988 (6, 14). SUS provides the Brazilian people the right to universal and free health care, from primary care to highly complex procedures. In addition, to reduce local and regional inequality, Brazil was divided into 438 health regions to correct these distortions among the States in order to optimize the distribution of resources and efficiency in the health care network (14). Health regions are delimited by an aggregate of several municipalities that ensure comprehensive healthcare assistance to the population within the region. Larger municipalities are responsible to provide any scale of care complexity and handle other administrative and technological complexities that can be used by citizens of other cities in the same region. Therefore, the citizens of a small town requiring a more complex service will resort to the services offered by a larger city within a given healthcare region (15).

Setting

Demographic and Socioeconomic Information

Demographic and socioeconomic indicators from Brazil were extracted from the Brazilian Institute of Geography and Statistics (IBGE, acronym in Portuguese) (16). Brazil occupies 8,510,295 km² in South America, and the estimates for the resident population in the 5,570 municipalities are 210,147,125 inhabitants. The income level of each municipality was based on the protocols used by the World Bank income classification (**Figure 1**) (17).

Health Care Facilities Infrastructure

The first step to verify the access of the population to ICU beds in Brazil was to geolocate all centers with ICU beds in Brazil. The Brazilian Health System involves public and private providers performing an extensive network of health care services (18). According to the Brazilian Society of Intensive Care (AMIB), the system comprises 8,011 health establishments, in which 1,961 have intensive care units, and 6,050 establishments

can perform only low complexity care (19). Therefore, not all municipalities have high-complexity centers (HCC), especially in remote regions disconnected from the network that provides emergency care services (ECS) (18). Thus, the population that requires high-complexity health procedures frequently needs to be referred to larger municipalities (18). The list of establishments with ICU beds was retrieved from the National Register of Health Facilities (CNES acronym in Portuguese) (20), and their location was gathered using R software with the package ggmap.

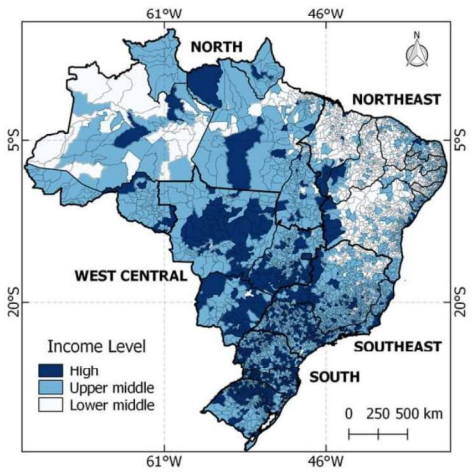


FIGURE 1 | Location of Brazil and distribution of national income for each municipality.

The Recommended Number of Professionals and Equipment

To evaluate the number of professionals and equipment available, we used the recommendations of the Resolution of the Collegiate Board, created by The National Health Surveillance Agency (ANVISA, acronym in Portuguese). The ANVISA is an agency subordinate to the Ministry of Health and responsible for the quality health regulations and services of the country. The Resolution of the Collegiate Board N°7 demands a qualified multidisciplinary team in the intensive care unit, composed of (a) one intensivist for every 10 beds; (b) one nurse for every eight beds; (c) one nursing technician per bed; and (d) one physiotherapist for every 10 beds (21). In the infirmary, the recommendations are (e) one physician per 10 beds; (f) one nurse per 10 beds; (g) one nursing technician per two beds; and (h) one physiotherapist for every 10 beds (21). Lastly, two ventilators per bed are required (21).

TABLE 1 | Data source, variables, date, and data used to develop this research in Brazil, 2020.

Source	Variables	Date range	Data entries	Reference
COVID-19 surveillance	Number of cases; Number of deaths.	June 2020	Confirmed cases and deaths by COVID-19	(22)
CNES National registration of health establishments	Limits of regional health entities; Number of ICU beds; Number of infirmary beds; Number of ventilators; Number of health professionals (intensivists, physicians, intensive care nurses, nurses, nursing assistants, and physiotherapists); Number of new adult and pediatric ICU beds exclusively for COVID-19.	February to June 2020	**February** 438 regional health entities 35,682 ICU beds; 426,388 infirmary beds; 65,411 ventilators; 18,716 intensivists; 564,529 Physicians; 2,768 intensive care nurses; 263,315 nurses; 710,846 nursing technicians; and 83,040 physiotherapists; **June** 17,240 new ICU beds exclusively for COVID-19.	(20)
IBGE Brazilian institute of geography and statistics	Population; Cartographic maps;	Estimate in 2019	Brazil: 210,147,125 inhabitants; North: 18,430,980; Northeast: 57,071,654; Midwest: 16,297,074; Southeast: 88,371,433; South: 29,975,984; Shapefiles for the country, states and regions.	(16)
World bank	Income level	2013	Income level for each municipality	(17)

Data Sources and Variables

All data on infrastructure and human resources were collected from the National Register of Health Facilities (CNES, acronym in Portuguese) (20). Information about COVID-19 cases was collected from the Secretariat of Surveillance of the Ministry of Health (22). Additional data were collected from IBGE databases (see **Table 1** for all datasets) (16). All data in CNES, COVID-19, and IBGE are freely available through an open data access platform.

Data Analysis

A comparison between recommendations with the actual numbers was performed for the numbers of professionals, equipment, and the distribution of health facilities to assess the adequacy of the Brazilian HCC network. The accessibility to establishments with ICU beds was analyzed based on the two-step floating catchment area (2SFCA) (23). The accessibility index resulting from the 2SFCA was assessed to highlight regions of the country facing lack of access to ICU.

Distribution of ICU and Hospital Beds and Professionals

The number of health professionals and equipment was extracted from the CNES database. The results were summed and divided by the number of inhabitants into each of the five administrative regions of Brazil (North, Northeast, Midwest, Southeast, and South).

Resources According to Standards

The number of ICU beds and infirmary beds was divided by the number of professionals and ventilators within the same health region, aiming to assess whether the resources comply with the standards in each health region defined by ANVISA. Then, the results were plotted on choropleth maps. The maps were performed using software QGIS version 3.16.6.

Spatial Distribution of COVID-19 Cases and Emergency Care Accessibility

The number of cases and mortality of COVID-19 was summarized and plotted in choropleth maps. The analysis of geographical accessibility to ICU beds was performed using the 2SFCA (23). The 2SFCA creates an index of accessibility, considering the geographical area weighted by population within the region. The first step is to create a radius of 120 km surrounding each HCC to verify the number of people most likely to be covered within a traveling distance of 2 h driving at 60 km/h (18). The second step creates a ratio (index) dividing the number of ICU beds by the number of people covered within the radius. Therefore, the accessibility index refers to the availability of ICU beds per population in each municipality. For this study, two accessibility indexes were created. The first one included regular ICU beds, which verifies the accessibility to HCC in February 2020, when Brazil did not report any official COVID-19 cases. The second refers to the access to new ICU beds by June 2020, when Brazil faced a high number of critical cases. Finally, a hotspot analysis was performed using Getis-Ord Gi* in ArcMap software version 10.5 to identify regions with significant clustering access to ICU beds. This analysis uses the

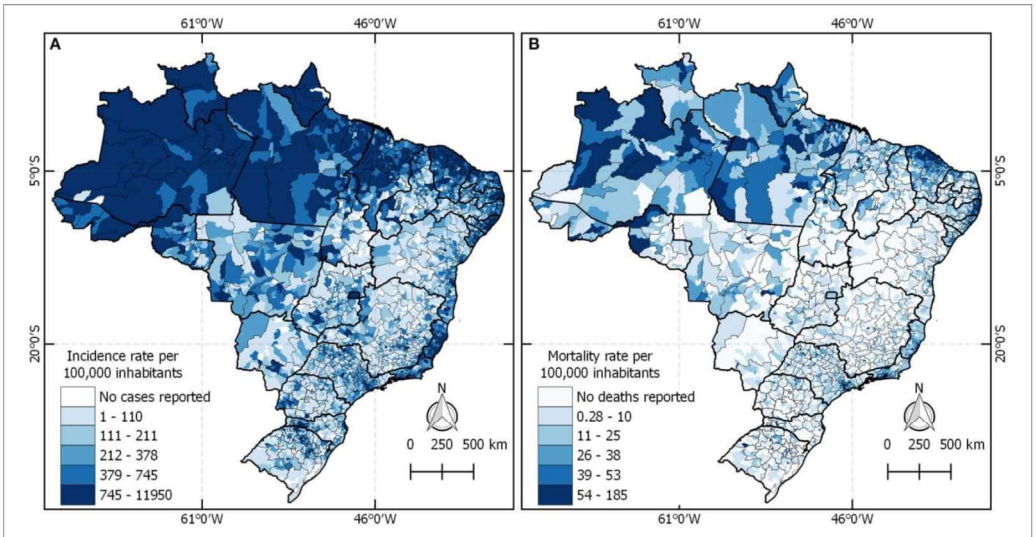


FIGURE 2 | The COVID-19 incidence and mortality rate in Brazil.

accessibility index and the incidence of COVID-19. It is possible to verify whether the formation of clusters in regions with access to health services is located within regions with a high incidence of the disease.

Ethical Approval

This study did not require ethical approval because no patient information was accessed, and all data are in the public domain (Resolution 510/2016 of the National Health Council).

RESULTS

COVID-19 in Brazil

Panel A in **Figure 2** depicts the COVID-19 incidence by Brazilian municipality up to June 2020. The darker colors indicate higher levels of COVID-19 cases or mortality. It is possible to observe that all states and regions were presenting COVID-19 cases, especially the states of Amazonas, Amapá, Espiríto Santo, and Santa Catarina. In terms of deaths, all state capitals in the north, northeast, and southeast regions presented high levels of mortality compared with the other states in the country.

Resources

As of February 2020, Brazil had 35,682 ICU beds, 426,388 hospital beds, and 65,411 ventilators. Additionally, Brazil has registered 18,716 intensivists, 564,529 general physicians, 2,768 intensive care nurses, 263,315 nurses, 710,846 nursing technicians, and 83,040 physiotherapists. The southeast region had the highest per population ICU beds, ventilators, physicians, nurses, and technicians, while the north region had the lowest (**Table 2**).

Figure 3 shows the distribution of professionals, beds, and ventilators across the country and classifies this distribution according to the recommendations. Blue regions on the map indicate that the amount of the analyzed resource is in accordance with the norms, while red regions show that the region is in

TABLE 2 | Rates of different types of bed and health professionals per population in each region in Brazil* (beds or health professionals/10,000 inhabitants).

Region	ICU beds	Infirmary beds	Physicians	Nurses	Nursing technicians	Ventilators
North	0.95	16.47	12.74	8.82	28.87	1.91
Northeast	1.19	20.00	17.60	11.62	27.39	2.19
Midwest	2.08	22.64	23.85	9.90	33.17	3.78
Southeast	2.11	19.46	34.70	14.14	38.57	3.82
South	1.70	24.33	31.72	13.21	35.46	3.13
Brazil*	1.70	20.29	26.86	12.53	33.82	3.11

*The Brazilian Institute of Geography and Statistics (IBGE) estimates that Brazil has 210,147,125 inhabitants.

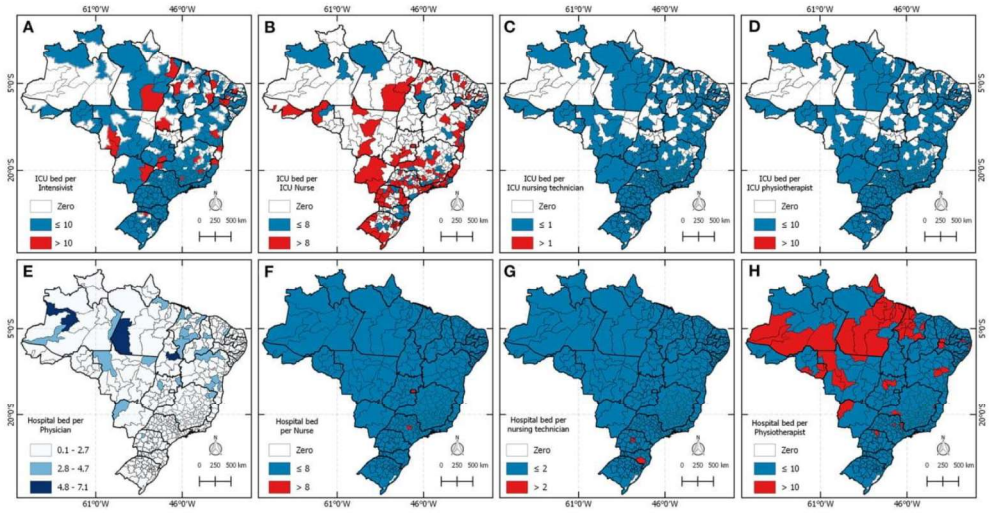


FIGURE 3 | Health regions in Brazil where the conditions of beds, equipment, and professionals are demonstrated according to ANVISA recommendations.

disagreement. In A, a large part of the national territory has intensivists working as recommended by ANVISA. However, 132 health regions do not have intensivists or do not have ICU beds, and 24 regions are operating beyond the recommendations. In B, only 53 regions are operating accordingly, 267 regions do not have ICU beds or intensive care nurses, and 117 are operating above the limit. Panels C and D show that most regions operate with the recommended number of nursing assistants

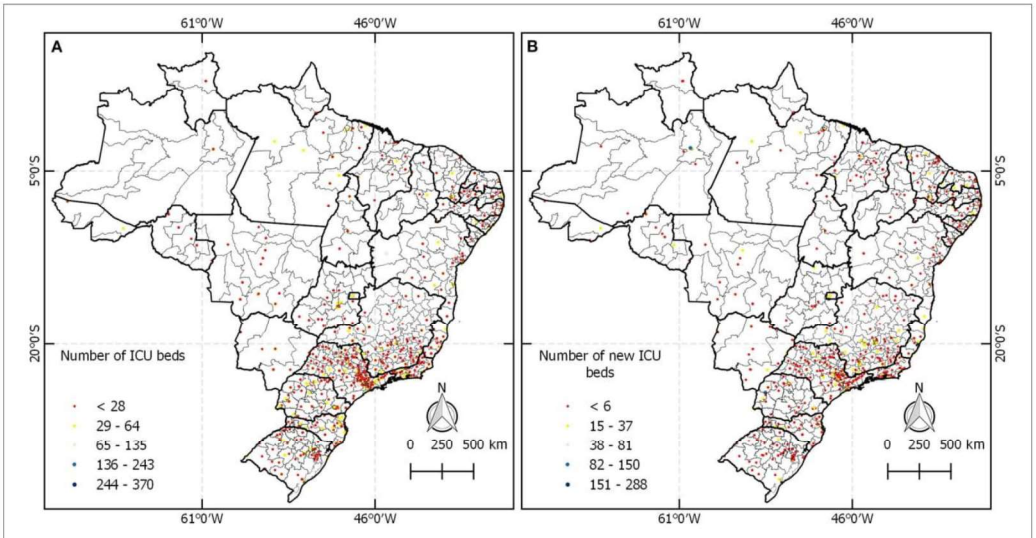


FIGURE 4 | (A) Location of beds of regular intensive care units. **(B)** Distribution of new ICU beds.

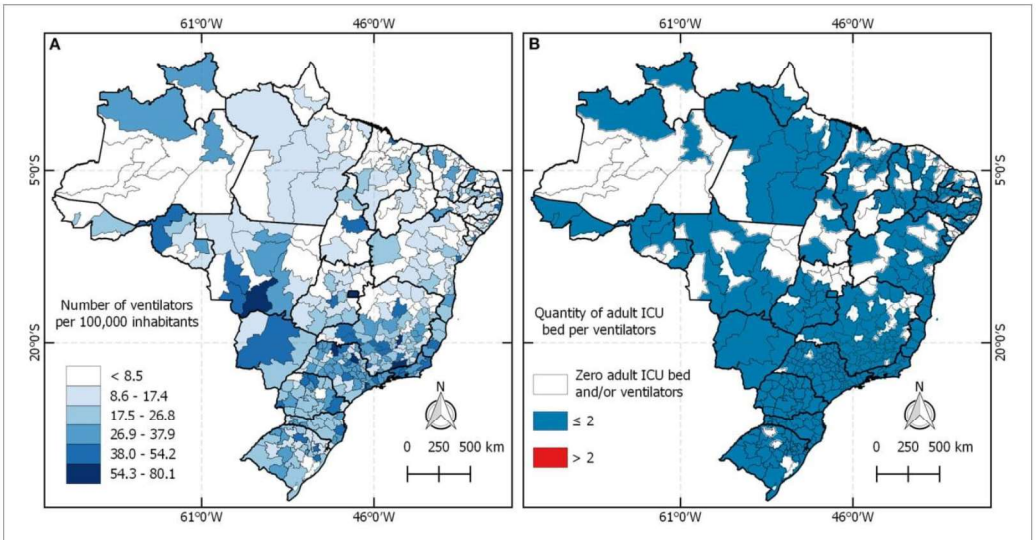


FIGURE 5 | (A) Distribution of ventilators in Brazil for every 10,000 inhabitants. **(B)** Ventilators per bed according to ANVISA.

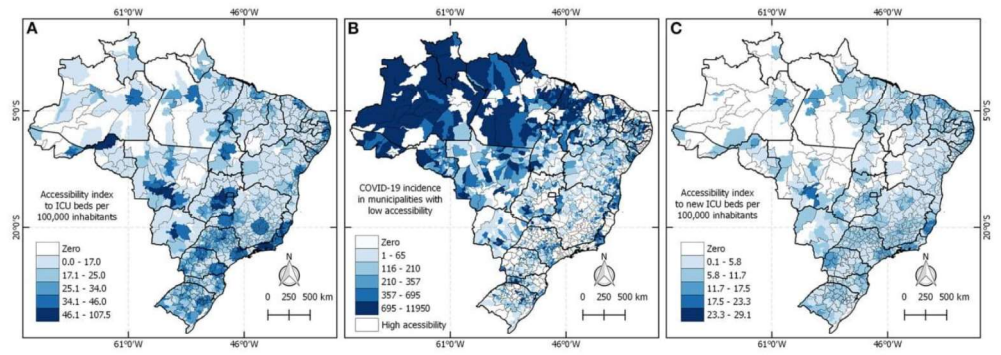


FIGURE 6 | Brazilian HCC network to tackle COVID-19 in 2020. **(A)** Shows the accessibility index in February. **(B)** Presents the disease incidence in the regions that lack HCC. **(C)** Exhibits the accessibility index of new ICU beds created to tackle COVID-19.

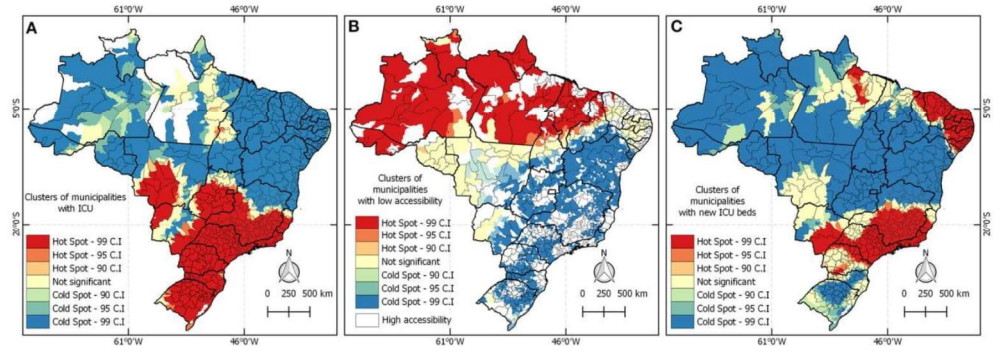


FIGURE 7 | Spatial clusters of access to ICU beds dedicated to tackling COVID-19 in 2020 in Brazil. **(A)** Shows the accessibility index in February 2020. **(B)** Presents the high incidence of the disease and the lack of emergency care services. **(C)** Exhibits clusters of accessibility index of new beds created until June 2020.

and physiotherapists in the ICU, and have regions where there are neither such professionals nor beds. In E, all regions of the country operate with an adequate number of physicians in the infirmary. However, it is still possible to observe that nurses, nursing assistants, and physiotherapists are taking care of infirmary beds beyond the preconized.

The panel A in **Figure 4** shows the distribution of all 35,682 regular intensive care beds in Brazil. Panel B shows 17,260 new ICU beds created until June 2020, where it is observed that most of the new ICU beds were located in the south and southeast regions, while the north region created many fewer.

Figure 5 presents the distribution of ICU beds per ventilator and shows that there are ICU beds in health regions in the north without ventilators available, or there are neither ICU beds nor ventilators.

Accessibility

Figure 6 presents three maps characterizing the access to HCC (establishments with ICU beds) in Brazil. Panel A shows the accessibility index to ICU beds per population and highlights a higher accessibility index in the Brazilian State capitals. Map B shows COVID-19 incidence only in municipalities where the accessibility index is below the national mean of 21.72 ICU beds per 1,000 inhabitants. Therefore, every municipality highlighted in map B has challenges in terms of HCC and high COVID-19 incidence, especially those municipalities in darker colors because they have a high incidence of COVID-19 and lack access to ICU beds. Map C presents the accessibility index to new ICU beds only, in which the results corroborate with the previous figures where the north region has lower accessibility to ICU beds in comparison to the rest of Brazil.

Figure 7 shows the result of spatial clustering analysis based on the indexes presented in **Figure 6**. Panel A exhibits clusters in midwestern, southeast, and south regions. Regions covered by the red layer represent the spatially significant group of municipalities with higher access to ICU beds. On the opposite side, the blue layer highlights the regions facing barriers of accessibility. Only in B, the red color covering municipalities in the north and northeast regions indicates regions of high-incidence COVID-19 and low numbers of ICU beds. Panel C illustrates the cluster of accessibility to new ICU beds. The red hotspots on maps B and C did not overlap, meaning that the new ICUs were not created in the regions that need them most.

DISCUSSION

Our study showed that Brazil has significant regional disparities in infrastructure and professional resources that affect health outcomes during COVID-19 pandemic. In general, our findings demonstrated a health care system suffering from geographical inequalities (6). However, the North region was the most affected by the pandemic due to the dispersion of cases of COVID-19, as demonstrated by Castro et al. (24), and corroborated with our study showing the lack of access to hospital resources.

The oxygen crisis in Manaus can be considered as an example of the consequences of the inequity distribution of emergency resources (25). During the first wave of COVID-19, around May 2020, Amazonas also experienced an exponential increase of deaths at home that led to a collapse of both the health care and funerary systems (25). Despite the consequences associated with the first wave, Manaus decided to relax social-distancing measures. When the second COVID-19 wave struck, oxygen shortages led to the deaths of up to 40 COVID-19 ICU patients in Manaus, in January 2021 (25). Key failures at the local and national levels contributed to these tragedies.

In Brazil, health professionals are not evenly distributed geographically to accomplish the Collegiate Board Recommendations Resolution, especially those who work in ICU. In terms of nursing and physicians in ICU, many regions have few critical care workers. These professionals care for more beds than recommended, potentially putting patients at risk of adverse outcomes (26). Kerlin et al. (27) verified that overworked professionals in critical care are more likely to experience burnout and provide unsatisfactory quality of care, which may have significant consequences for patient care and the healthcare system. In addition, the intense and emotionally charged work drains healthcare providers physically and emotionally to the point where they may get sick, further limiting their ability to treat patients and reducing the available workforce (28). Therefore, Brazilian regions with overworked professionals may face a more significant challenge against COVID-19.

Health resources in Brazil are majorly accessible to the people living in urban areas, with abundant access focused in larger centers in the southeast and south regions such as São Paulo, Rio de Janeiro, Paraná, and Santa Catarina States. On the other hand, underdeveloped, rural, and sparsely populated regions did not receive the same attention in comparison to the developed areas. The uneven distribution of health resources led to a disproportionate number of cases and mortality of COVID-19.

Here we analyzed the distribution of ICU beds in February, before the arrival of COVID-19 in Brazil, to verify the preparation for health resources management. Initially, few hospital beds were relocated to serve only patients with COVID-19, which reduced the number of beds available for patients with other pathologies. This maneuver affected negatively densely populated regions where they usually have a greater availability of beds. However, the impact was even worse in regions with few beds. Therefore, to circumvent this situation, new ICU beds were created to serve patients with COVID-19, apart from preexisting ones. But when the first wave hit Brazil by June, we verified that new beds were mainly added in the south and southeast regions, where there were already higher numbers of beds in comparison to other regions. Thus, those regions with few beds faced a disproportional challenge to overcome COVID-19.

In Brazil, existing socioeconomic inequalities have affected the course of COVID-19, with a disproportionate adverse burden on states and municipalities with high socioeconomic vulnerability (29). The results we got are aligned with other studies in the literature, emphasizing that the distribution of emergency resources dedicated to tackle COVID-19 followed the patterns of inequalities present in the country before the pandemic (29). Part of this outcome was due to a lack of central coordination to better drive the opening of new ICU beds, distribution of ventilators, as well as the other resources to support emergency care. The political context posed a major challenge to the response. The risk of the virus has been downplayed by the current political leadership in Brazil, and messages from the federal government have been mixed with regard to physical distancing restrictions, use of face masks, and reopening plans, among other aspects (29).

The surge of critical supply shortages affected all countries and led to a worldwide race for ventilators (30). The availability of ventilators in Brazil was not satisfactory in several regions, especially in the north and northeast regions. Therefore, the most severe cases of COVID-19 may be more overwhelming in these regions (31). This situation predicted and stated that Brazil faced a double crisis: COVID-19 and internal political conflicts. The federal government went against the Ministry of Health and WHO's technical recommendations when COVID-19 reached Brazil (32). Thus, each administrative level implemented isolated responses to prepare for COVID-19 with limited country-level coordination. The effective absence of a Brazilian government response to COVID-19 made the country particularly susceptible to outcome inequities (33). Furthermore, healthcare has been overlooked by the government, delaying and promoting insufficient resources to aid the most vulnerable population (34).

This study presents its strengths. First, all data are publicly available. In addition, this is one of the first studies to perform spatial analysis to verify the uneven distribution of ICU beds in Brazil. However, the study also presents some limitations. Biases are commonly found in observational studies based on secondary data and cannot be ruled out, especially here since the data were obtained from the government. The accessibility index to ICU beds was performed, taking

into consideration that everyone within the radius of 120 km from HCC had the same chance to be treated. However, the geography in Brazil is highly diverse, and the population in the Amazon region probably had higher challenges since their transportation frequently relies on rivers. Furthermore, we could be overestimating the accessibility of large centers because patients deliberately travel great distances to larger centers to have a greater chance of being assisted, overloading this center as it would be serving a population outside the scope stipulated by the analysis.

In conclusion, Brazil has a myriad of infrastructure challenges limiting its ability to treat critically ill patients. We have demonstrated an insufficient number of ICU beds and ventilators, and a severely limited number and inequitable healthcare professional distribution. Additionally, the allocation of new beds did not address areas with known gaps in care and health infrastructure accessibility. Therefore, the SARS-CoV-2 epidemic in Brazil may be devastating, especially in areas with limited health infrastructure. Lastly, this study suggests that strong leadership is needed to coordinate the response efforts against COVID-19.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

LS contributed to the investigation, software, formal analysis, and writing—original draft. AC, PI, and GR made substantial contributions to the data curation and formal analysis. LA, IP, JC, PV, and TH performed the methodology, formal analysis, validation, and visualization. CS contributed to the supervision and writing—review and editing. JN and RF contributed equally to the conceptualization, project administration, and contributed to the supervision and writing—review and editing. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

We would like to thank the Coordination for the Improvement of Higher Education Personnel (CAPES) Code 001.

REFERENCES

1. Sohrabi C, Alsafi Z, O'Neill N, Khan M, Kerwan A, Al-Jabir A, Iosifidis C, Agha R. World health organization declares global emergency: a review of the 2019 novel coronavirus (COVID-19). *Int J Surg.* (2020) 76:71–76. doi: 10.1016/j.ijsu.2020.02.034
2. World Health Organization. COVID-19 Weekly Epidemiological Update-−6 July 2021 (2021). Available online at: https://www.who.int/publications/m/item/weekly-epidemiological-update-on-covid-19 (accessed July 10, 2021).
3. de Oliveira Andrade R. Covid-19 is causing the collapse of Brazil's national health service. *BMJ.* (2020) 11:m3032. doi: 10.1136/bmj.m3032
4. Communication. collaboration and cooperation can stop the 2019 coronavirus. *Nat Med.* (2020) 26:151–151. doi: 10.1038/s41591-020-0775-x
5. Phua J, Weng L, Ling L, Egi M, Lim C-M, Divatia JV, et al. Intensive care management of coronavirus disease 2019 (COVID-19): challenges and recommendations. *Lancet Respirator Med.* (2020) 8:506–517. doi: 10.1016/S2213-2600(20)30161-2
6. Castro MC, Massuda A, Almeida G, Menezes-Filho NA, Andrade MV, de Souza Noronha KVM, et al. Brazil's unified health system: the first 30 years and prospects for the future. *Lancet.* (2019) 394:345–356. doi: 10.1016/S0140-6736(19)31243-7
7. Donida B, da Costa CA, Scherer JN. Making the COVID-19 pandemic a driver for digital health: Brazilian strategies. *JMIR Public Health Surv.* (2021) 7:e28643. doi: 10.2196/28643
8. Paim J, Travassos C, Almeida C, Bahia L, Macinko J. The brazilian health system: history, advances, and challenges. *Lancet.* (2011) 377:1778–1797. doi: 10.1016/S0140-6736(11)60054-8
9. Liu S, Wang Y, Zhou D, Kang Y. Two-step floating catchment area model-based evaluation of community care facilities' spatial accessibility in Xi'an, China. *IJERPH.* (2020) 17:5086. doi: 10.3390/ijerph17145086
10. de Carvalho Dutra A, Silva LL, Pedroso RB, Tchuisseu YP, da Silva MT, Bergamini M, et al. The impact of socioeconomic factors, coverage and access to health on heart ischemic disease mortality in a Brazilian Southern State: a geospatial analysis. *Glob Heart.* (2021) 16:5. doi: 10.5334/gh.770
11. Higgs G, Langford M, Jarvis P, Page N, Richards J, Fry R. Using geographic information systems to investigate variations in accessibility to 'extended hours' primary healthcare provision. *Health Soc Care Commun.* (2019) 27:1074 84. doi: 10.1111/hsc.12724
12. Ngui AN, Apparicio P. Optimizing the two-step floating catchment area method for measuring spatial accessibility to medical clinics in Montreal. *BMC Health Serv Res.* (2011) 11:166. doi: 10.1186/1472-6963-11-166
13. Myles PS, Maswime S. Mitigating the risks of surgery during the COVID-19 pandemic. *Lancet.* (2020) 396:2–3. doi: 10.1016/S0140-6736(20)31256-3
14. Brasil. *Decreto n° 7.508, de 28 de junho de 2011. Regulamenta a Lei no 8.080, de 19 de setembro de 1990, para dispor sobre a organização do Sistema Único de Saúde-SUS, o planejamento da saúde, a assistência à saúde e a articulação interfederativa, e dá outras providências. Diário Oficial da União 1.* (2011). Available online at: http://www.planalto.gov.br/ccivil_03/_ato2011-2014/2011/decreto/d7508.htm (accessed May 12, 2020).
15. Santos L. Região de saúde e suas redes de atenção: modelo organizativo-sistêmico do SUS. *Ciênc saúde coletiva.* (2017) 22:1281–1289. doi: 10.1590/1413-81232017224.26392016
16. Brasil. *IBGE. Instituto Brasileiro de Geografia e Estatística. Séries estatísticas & séries históricas. Rio de Janeiro.* (2020). Available online at: https://www.ibge.gov.br/ (accessed May 1, 2020).
17. Fantom NJ, Serajuddin U. *The World Bank's classification of countries by income. The World Bank.* (2016). Available online at: http://documents.worldbank.org/curated/en/408581467988942234/The-World-Banks-classification-of-countries-by-income (accessed April 10, 2020).
18. Rocha TAH, da Silva NC, Amaral PV, Barbosa ACQ, Rocha JVM, Alvares V, et al. Access to emergency care services: a transversal ecological study about brazilian emergency health care network. *Public Health.* (2017) 153:9–15. doi: 10.1016/j.puhe.2017.07.013
19. Brasil. *Associação Brasileira de Medicina Intensiva.* (2020). Available online at: https://www.amib.org.br/censo-amib/censo-amib-2016/ (accessed May 15, 2020).
20. Saldanha R de F, Bastos RR, Barcellos C. Microdatasus: pacote para download e pré-processamento de microdados do Departamento de Informática do SUS (DATASUS). *Cad Saúde Pública.* (2019) 35:e00032419. doi: 10.1590/0102-311x00032419
21. Brasil. *Agência Nacional de Vigilância Sanitária. RDC. N° 7, de 24 de fevereiro de 2010. Dispõe sobre os requisitos mínimos para funcionamento de Unidades de Terapia Intensiva e dá outras providências Brasília.* (2020). Available online at: https://bvsms.saude.gov.br/bvs/saudelegis/anvisa/2010/res0007_24_02_2010.html (accessed April 12, 2020).

22. Brasil em dados libertos. *Repositório de dados públicos disponibilizados em formato acessível.* (2020). Available online at: http://brasil.io (accessed May 20, 2020).
23. Wang F, Luo W. Assessing spatial and nonspatial factors for healthcare access: towards an integrated approach to defining health professional shortage areas. *Health Place.* (2005) 11:131–146. doi: 10.1016/j.healthplace.2004.02.003
24. Castro MC, Kim S, Barberia L, Ribeiro AF, Gurzenda S, Ribeiro KB, Abbott E, Blossom J, Rache B, Singer BH. Spatiotemporal pattern of COVID-19 spread in Brazil. *Science.* (2021) 372:821–826. doi: 10.1126/science.abh1558
25. Malta M, Strathdee SA, Garcia PJ. The brazilian tragedy: where patients living at the 'earth's lungs' die of asphyxia, and the fallacy of herd immunity is killing people. *EClinicalMedicine.* (2021) 32:100757. doi: 10.1016/j.eclinm.2021.100757
26. Zhang Y, Wang C, Pan W, Zheng J, Gao J, Huang X, Cai S, Zhai Y, Latour JM, Zhu C. Stress, Burnout, and coping strategies of frontline nurses during the COVID-19 epidemic in wuhan and shanghai, China. *Front Psychiatry.* (2020) 11:565520. doi: 10.3389/fpsyt.2020.565520
27. Kerlin MP, McPeake J, Mikkelsen ME. Burnout and Joy in the Profession of Critical Care Medicine. *Crit Care.* (2020) 24:98. doi: 10.1186/s13054-020-2784-z
28. Liu Q, Luo D, Haase JE, Guo Q, Wang XQ, Liu S, Xia L, Liu Z, Yang J, Yang BX. The experiences of health-care providers during the COVID-19 crisis in China: a qualitative study. *The Lancet Global Health.* (2020) 8:e790–e798. doi: 10.1016/S2214-109X(20)30204-7
29. Rocha R, Atun R, Massuda A, Rache B, Spinola P, Nunes L, Lago M, Castro MC. Effect of socioeconomic inequalities and vulnerabilities on health-system preparedness and response to COVID-19 in Brazil: a comprehensive analysis. *The Lancet Global Health.* (2021) 9:e782–e792. doi: 10.1016/S2214-109X(21)00081-4
30. Ranney ML, Griffeth V, Jha AK. Critical Supply Shortages — The Need for Ventilators and Personal Protective Equipment during the Covid-19 Pandemic. *N Engl J Med.* (2020) 382:e41. doi: 10.1056/NEJMp2006141
31. Ranzani OT, Bastos LSL, Gelli JGM, Marchesi JF, Baião F, Hamacher S, Bozza FA. Characterisation of the first 250 000 hospital admissions for COVID-19 in Brazil: a retrospective analysis of nationwide data. *The Lancet Respiratory Medicine.* (2021) 9:407–418. doi: 10.1016/S2213-2600(20)30560-9
32. The Lancet. COVID-19 in Brazil: "So what?" *The Lancet.* (2020) 395:1461. doi: 10.1016/S0140-6736(20)31095-3
33. Baqui P, Bica I, Marra V, Ercole A, van der Schaar M. Ethnic and regional variations in hospital mortality from COVID-19 in Brazil: a cross-sectional observational study. *The Lancet Global Health.* (2020) 8:e1018–e1026. doi: 10.1016/S2214-109X(20)30285-0
34. Ferigato S, Fernandez M, Amorim M, Ambrogi I, Fernandes LMM, Pacheco R. The Brazilian Government's mistakes in responding to the COVID-19 pandemic. *The Lancet.* (2020) 396:1636. doi: 10.1016/S0140-6736(20)32164-4

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Artigo 2: “Enhancing COVID-19 Vaccination in South Brazil: a model based on rearranging distribution of vaccines to preexisting sites to increase coverage”

Enhancing COVID-19 Vaccination in South Brazil: a model based on rearranging distribution of vaccines to preexisting sites to increase coverage

Authors:

Lincoln Luís Silva[1]; Thiago Augusto Hernandez Rocha[2]; Luciano de Andrade[3]; Catherine Ann Staton[4]; João Ricardo Nickenig Vissoci[4]; Rosilene Fressatti Cardoso[5]

Affiliation:

[1] Post-Graduation Program in Biosciences and Physiopathology, State University of Maringá, Maringá, PR, Brazil;

[2] Pan American Health Organization (PAHO), Washington D.C, USA;

[3] Department of Medicine, State University of Maringa, Maringá, PR, Brazil;

[4] Department of Surgery, Duke University, Durham, North Carolina, USA;

[5] Department of Clinical Analysis and Biomedicine, State University of Maringá, Maringá, PR, Brazil.

Corresponding author:

Profa. Dra. Rosilene Fressatti Cardoso

rfcardoso@uem.br

Abstract

Background

SARS-CoV-2 is the infectious agent that causes COVID-19, a viral disease that devasted several health systems in the world. Despite measures were adopted to curb the spread, the vaccine is essential to overcome the disease. However, ensuring proper distribution of vaccine is complex, but geographical information system techniques may provide information to assist policy makers with this task. Therefore, this study provides substantial information on optimization of vaccine distribution using location-allocation.

Methods

Data from the National Immunization Program Information System in Brazil was collected to verify the number of people vaccinated and the location of immunization sites for COVID-19. Additionally, location of all health facilities authorized to administer vaccine were obtained from the National registry of health establishments to identify potential new sites for COVID-19 immunization. Finally, a spatial analysis utilizing the location-allocation method was conducted to determine the optimal number of facilities needed to maximize coverage in a population in Southern Brazil.

Results:

Between January 2021 and January 2022, the state of Paraná administered the first dose of a COVID-19 vaccine to 9,100,843 individuals, with the highest number of vaccinations occurring in June, July, and August of 2021. By December of 2021, nearly all municipalities in the state reached an immunization rate of 70%. A location-allocation model was utilized, which identified 56 new potential establishments that would increase population coverage by more than 250,000 people.

Conclusion:

The study verified the sociodemographic characteristics of individuals vaccinated against COVID-19 in Paraná and proposed that 56 additional Basic Health Units would increase population vaccination coverage in the state.

Keywords: COVID-19; SARS-CoV-2, public health, spatial analysis; epidemiology.

Introduction

The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is the causative agent of COVID-19 that caused the death of millions of people and brought the chaos the health care system globally (1). In 2022, more than six million deaths have already been reported (2), of which 677 thousand occurred in Brazil (3). In an effort to curb the spread of cases and ultimately reduce fatalities, several measures were adopted such as the use of masks, mass testing, and social distancing (4). Even though these measures did have some impact, only the widespread distribution of vaccines that appeared to be the most effective solution to the problem (4). With some COVID-19 vaccines now authorized for human use, the key challenge now is ensuring proper distribution to achieve control over the pandemic (5).

In addition, to achieve this goal, it is crucial that a significant proportion of the population is vaccinated, with estimates ranging from 70-75% depending on the vaccine's efficacy (6). Vaccination coverage, or the percentage of the target population that has been vaccinated, serves as an important indicator of the effectiveness of a country's health and immunization systems (7). Vaccination coverage stands out for the benefits it brings to socioeconomic determinants (8), such as: its contributions to increased children's school attendance and educational performance, increases in family income and, ultimately, greater national economic growth (9). However, achieving high vaccination coverage requires the collaboration and coordination of efforts from the federal government, healthcare professionals, and public managers through strategic planning.

Recently, some countries have shown interest in the use of Geographic Information Systems (GIS) to support the development of strategies that optimize vaccination sites (10, 11). The use of GIS tools has played a critical role in understanding the spatial clustering and transmission trend of COVID-19 (12), distribution of hospital beds (13), and is being instrumental in real-time case mapping (14). In addition, there are several approaches that can be used to optimize the implementation of vaccination sites in order to increase vaccine coverage, one of which is location-allocation (15), in which the spatial technique seeks to maximize the population that can be served within a limited distance for a number of service locations (15). Thus, GIS tools provide a theoretical and practical foundation that can assist public health managers in effectively allocating resources to serve the population.

Given the current COVID-19 situation in Brazil, the urgent need for vaccination of the population, and the potential of GIS tools for public health, strategic and coordinated planning is essential to ensure that vaccination is carried out in an efficient and effective manner. To that

end, this study proposes a geospatial analysis to determine the optimal number of healthcare facilities required to reach the largest number of individuals eligible for vaccination against COVID-19 in the state of Paraná in southern Brazil.

Methods

Study design

This is an ecological study with secondary data from people vaccinated against COVID-19 in Paraná - Brazil, in order to identify the location of existing vaccination sites to evaluate the feasibility of adding new sites to improve vaccination coverage across the state using location-allocation spatial analysis.

Population study

All persons vaccinated with the first dose of COVID-19 vaccine in the state of Paraná who are registered in the National Immunization Program Information System (SI-PNI) were included in this study. According to the Brazilian Institute of Geography and Statistics (IBGE), the state of Paraná is located in the southern region of Brazil, with a territorial area of 199 thousand km², divided into 399 municipalities, 22 health regions, and an estimated population of 11.5 million inhabitants (16).

Vaccination sites

In Brazil, individuals eligible for vaccination are assisted at Basic Health Units (BHU), referred as primary healthcare network, in which are locals specifically equipped with facilities for administering vaccines (17). These facilities are also registered in the National Registry of Health Establishments (CNES) and their identification numbers are recorded in each patient's vaccination records, allowing for easy identification of the location where each individual received their vaccination.

Spatial analysis

To determine the optimal number of Basic Health Units (BHU) required to serve the largest number of people in the state of Paraná, this study utilized a four-step approach: first, the locations used for vaccination were identified and geolocated, then the distribution of the

129 population was analyzed, the transportation network was extracted to assess the accessibility of
130 the BHUs, and finally, a location-allocation analysis was conducted to identify potential new
131 sites that can increase vaccination coverage. This approach takes into account the spatial
132 distribution of population, accessibility of transportation and availability of resources to
133 identify the best location for new vaccination sites.
134
135 Geolocation of vaccination sites
136 The CNES number of the place where the person was vaccinated was accessed on the
137 CNES website (https://cnes.datasus.gov.br/) to obtain the street and municipality to which that
138 establishment belongs. Then, the geographic coordinates were obtained using *ggmap* package
139 in R software with a Google Geocoding API.
140
141 Population distribution
142 The population distribution in Paraná was estimated using Dasymetric modeling from
143 Worldpop in 2021 (18). WorldPop is a project that provides detailed population data for
144 countries around the world with combination of several data sources, including satellite
145 imagery, national censuses, and survey data to create high-resolution population maps. The data
146 is then made available through an open-access platform, allowing for easy access and use by
147 researchers (18). Dasymetric analysis is a technique used in GIS to verify a more accurate
148 representation of population distribution, which can be useful in a variety of applications such
149 as urban planning, emergency management and health research (19). In this study, we then
150 performed a tessellation technique to verify the number of people inside of hexagons with area
151 of 25 km^2, because hexagons are suitable for reducing sampling bias due to edge effects
152 compared to other shapes (20).
153
154 Transportation
155 In this work, the terrestrial transport network obtained using a plugin developed by
156 Rocha et al. (2020) that uses OSM in the background (21, 22), in which provides reliable
157 navigation containing maximum street speed, and direction, for example. OpenStreetMap, also
158 known as OSM, is an open-source project with contributors from all over the world who add
159 and update map data regularly, meaning that it can be used, distributed, and modified freely.
160 (21). The data is collected through GPS, aerial imagery, and community contributions and is

made available through an API, making it easy to integrate into other applications and services (21). OSM is widely used in various fields such as GIS, cartography, transportation and more, especially in areas where commercial map data is not available or outdated.

Location-allocation

In this study, we employed a location-allocation model to optimize the distribution of Basic Health Units (BHUs) in order to improve accessibility to healthcare services for the population. The model was configured to maximize attendance at the nearest BHU within a 7-minute travel time from the population, taking into account the transport network. The model utilizes a weighted distance minimization algorithm, assuming that the population will utilize the nearest facility (15). The optimization process was conducted until the coverage reached a plateau, indicating that adding more facilities would not result in any further improvement in coverage. In other words, the model was configured to optimize the distribution of BHUs by maximizing accessibility for the population, and it was stopped when further addition of facilities would not lead to a significant improvement of coverage.

Data collection

Our study aimed to analyze the distribution of vaccinated patients in the state of Paraná, Brazil. To accomplish this, we utilized data from several sources. First, we obtained data on vaccinated patients from the SI-PNI (https://dados.gov.br/), a database maintained by the Brazilian government that contains information on vaccination coverage. We also accessed information on health facilities in the state from the CNES website (https://cnes.datasus.gov.br/), a database maintained by the Brazilian Ministry of Health that provides information on healthcare facilities. The spatial distribution of the data was represented in maps created using shapefiles from the IBGE (16), a government agency responsible for producing geographic and demographic data. All of the data used in this study is publicly available and can be accessed without any restrictions.

Data analysis

The study population's socio-demographic characteristics were analyzed utilizing quantitative methodologies, specifically through the utilization of both absolute and relative frequency distributions using software R version 4.0. Additionally, spatial analysis was conducted via the use of ArcGIS Pro version 2.9, and figures and visualizations presented in

194 this study were generated using QGIS 3.18, a widely accepted and utilized open-source
195 software for geographic information systems.

196

197 Ethics

198 This study utilized secondary data as its primary source of information and did not
199 include any sensitive information. All data and information used in this research is publicly
200 available and therefore, did not require review or approval from a Research Ethics Committee.

201

202 **Results**

203 From January 2021 to January 2022, 9,100,843 people in Paraná were vaccinated with
204 the first dose of COVID-19 vaccine. In Paraná, four vaccines from different manufacturers were
205 used: Astrazeneca, representing the majority of those vaccinated with the first dose (38.70%),
206 Pfizer, SINOVAC/Butantan, Janssen. In addition, each of these vaccines represented a different
207 age group vaccinated, which can be explained by the time when a given vaccine was available
208 to be applied and the target population according to the vaccination schedule. Regarding gender,
209 there was a balance between male and female (52.57% vs 47.43%), as for race, most were white
210 (63.51%).

211

Table 1. Descriptive analysis of vaccinated people with the first dose against COVID-19 in Paraná, Brazil.

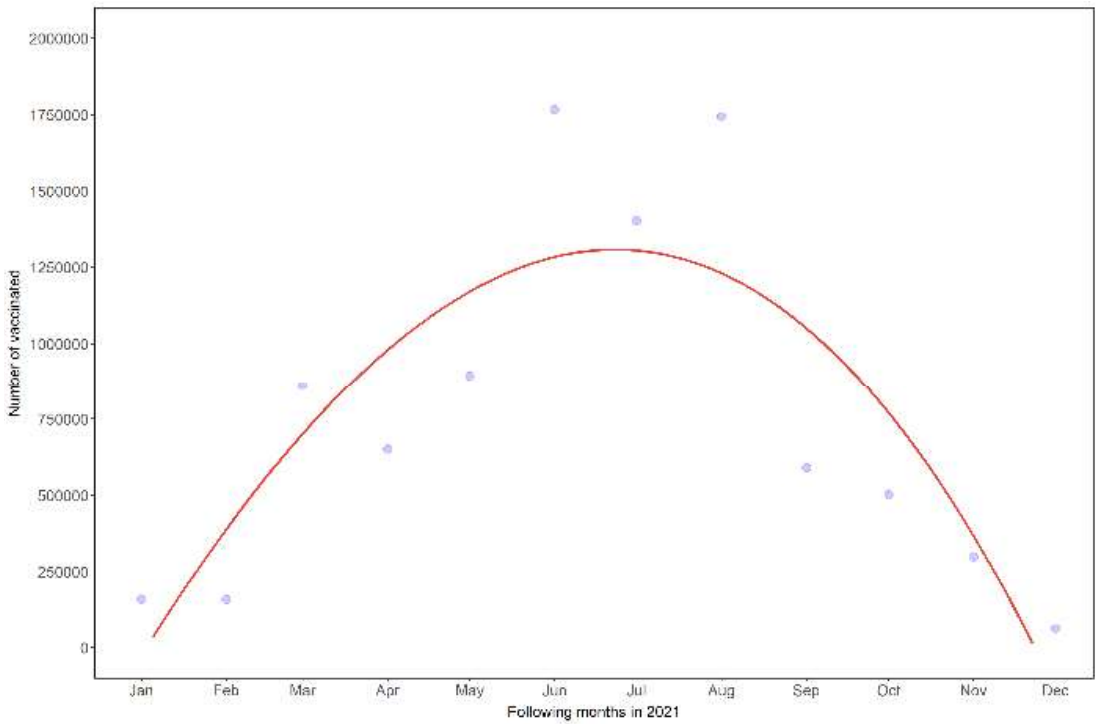
Characteristic	Overall N = 9,100,843 (100%)	Astrazeneca N = 3,522,094 (38.70%)	Janssen N = 858 (<0.01%)	Pfizer N = 3,379,762 (37.14%)	SINOVAC/Butantan N = 2,198,129 (24.15%)
Median age	40 (26, 56)	51 (39, 59)	38 (29, 46)	27 (18, 40)	46 (29, 71)
Sex					
Female	4,316,276 (47.43%)	1,630,274 (46.29%)	771 (89.86%)	1,687,159 (49.92%)	998,072 (45.41%)
Male	4,784,471 (52.57%)	1,891,799 (53.71%)	87 (10.14%)	1,692,541 (50.08%)	1,200,044 (54.59%)
Race					

Table 1. Descriptive analysis of vaccinated people with the first dose against COVID-19 in Paraná, Brazil.

Characteristic	Overall N = 9,100,843 (100%)	Astrazeneca N = 3,522,094 (38.70%)	Janssen N = 858 (<0.01%)	Pfizer N = 3,379,762 (37.14%)	SINOVAC/Butantan N = 2,198,129 (24.15%)
Yellow	460,903 (5.06%)	180,478 (5.12%)	50 (5.83%)	166,160 (4.92%)	114,215 (5.20%)
White	5,780,044 (63.51%)	2,243,942 (63.71%)	412 (48.02%)	2,130,838 (63.05%)	1,404,852 (63.91%)
Indigenous	11,766 (0.13%)	614 (0.02%)	0 (0.00%)	2,631 (0.08%)	8,521 (0.39%)
Brown	749,305 (8.23%)	274,141 (7.78%)	66 (7.69%)	298,027 (8.82%)	177,071 (8.06%)
Black	198,877 (2.19%)	80,619 (2.29%)	17 (1.98%)	66,556 (1.97%)	51,685 (2.35%)
Ignored	1,899,863 (20.88%)	742,282 (21.08%)	313 (36.48%)	715,494 (21.17%)	441,774 (20.10%)

Panel A in Figure 1 shows the monthly evolution of the number of people from Paraná vaccinated with the first dose of COVID-19 vaccine during the year 2021. It is possible to see that June, July and August were the months in which there were the highest number of vaccinated people. Panel B shows the number of people vaccinated by the municipality.

A



B

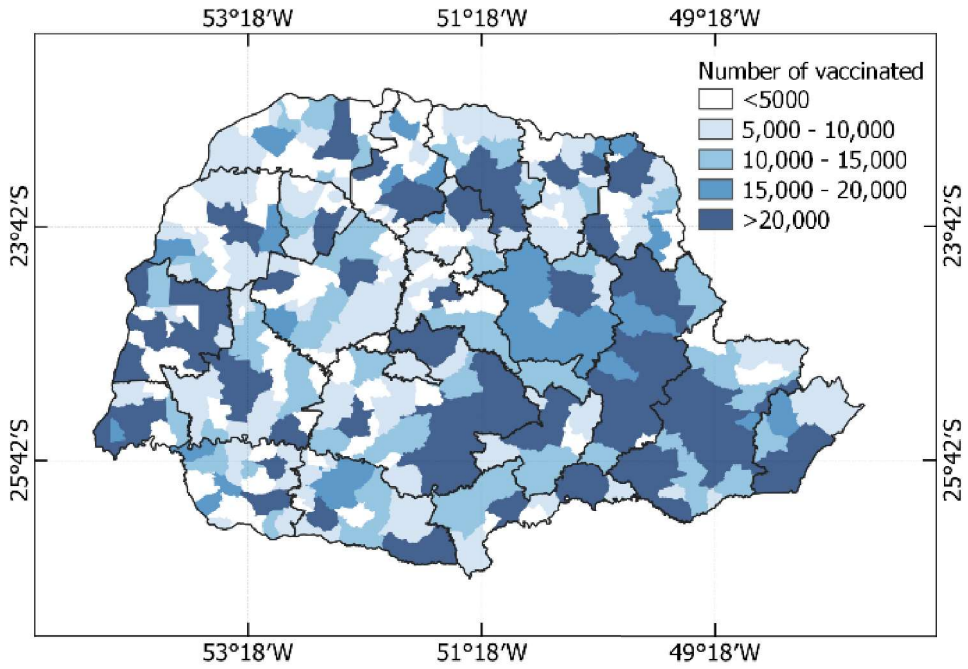
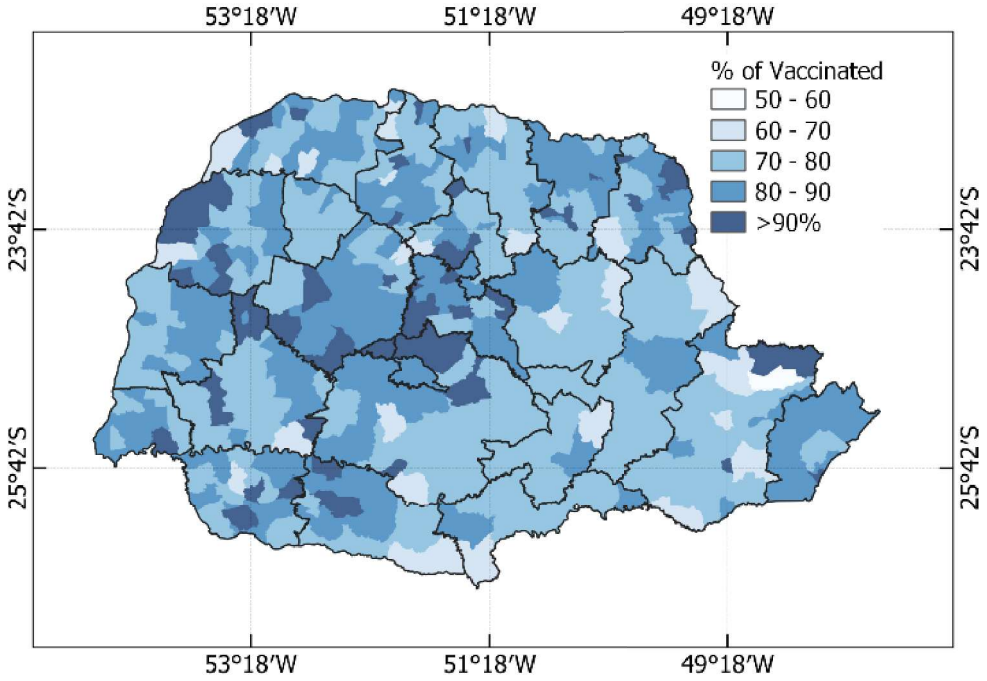


Figure 1. Timeline and number of vaccinated people with the first dose of COVID-19 in Paraná in 2021.

227

In Figure 2, All 399 municipalities in Paraná until the end of the study period had cases of first-dose vaccination (Panel A). However, only 27 (6.76%) of municipalities still had vaccination below 70% (Panel B). Curiously, 14 (3.50%) municipalities had more than 100% of their population vaccinated.

A



B

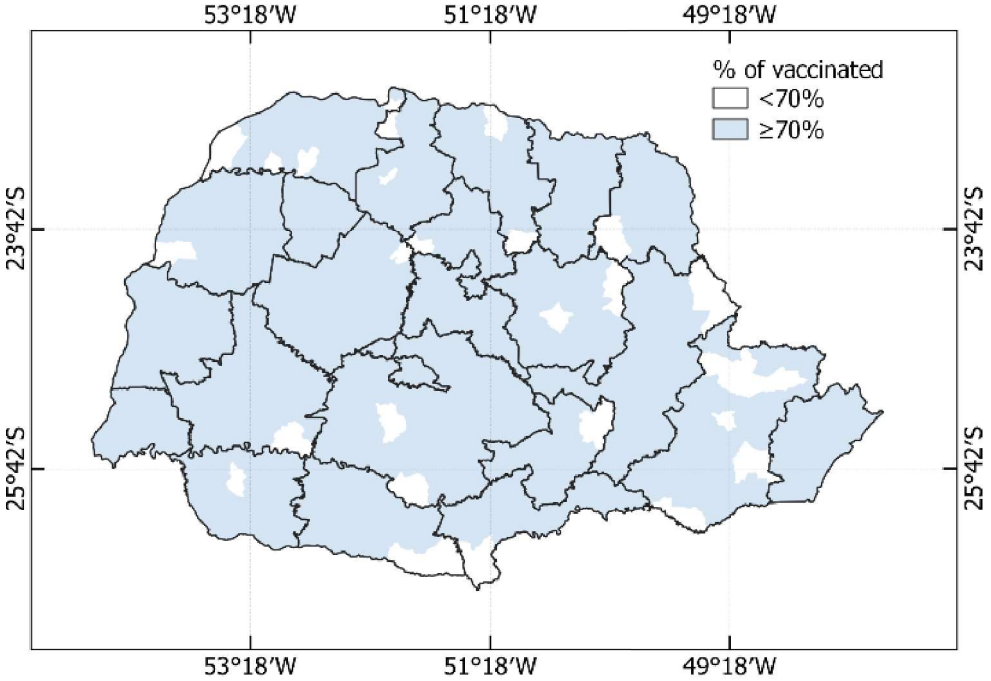
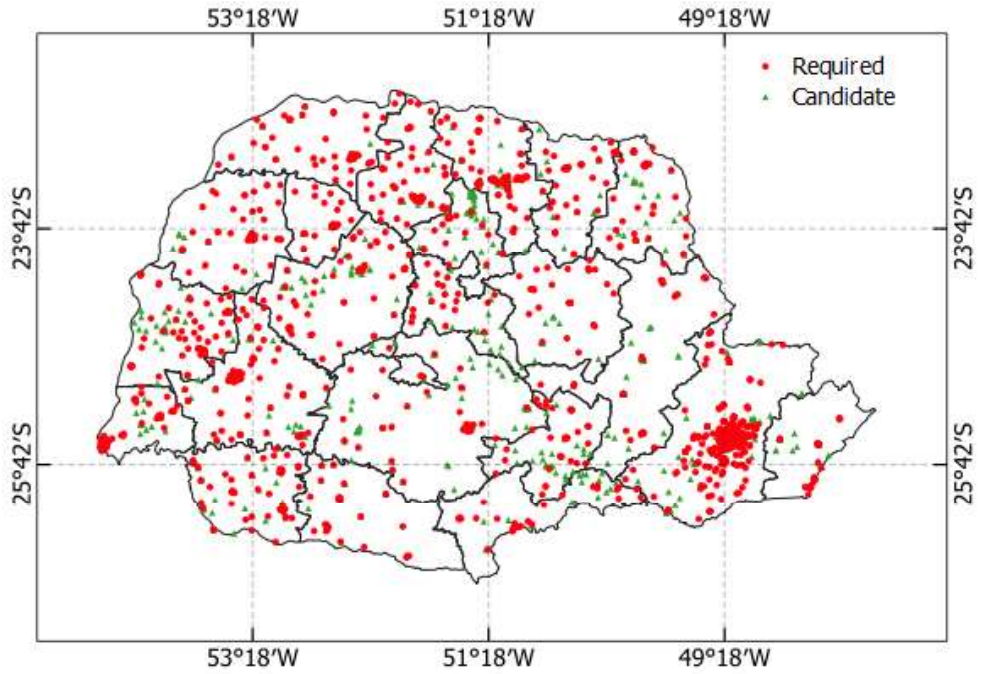


Figure 2. Percentage of people vaccinated with the first dose of COVID-19 and regions that are above the recommendations in Paraná in 2021.

Regarding vaccination sites, 1,269 health establishments were used. A total of 100 BHU out of 678 candidates were used to simulate the increase in vaccination coverage. In panel A of Figure 3, the red circles in panel A show the Basic Health Units used in the vaccination network, and the green triangle shape shows the possible strategic locations to increase vaccination coverage. In our model, 56 more BHU were enough to cover the maximum number population in Parana. Therefore, when adding these 56 potential establishments in the vaccination network, the population covered ranged from 35.84% to 37.69%, which represents a gain of 2.15% and covers 250,077 more people (Panel B).

A



B

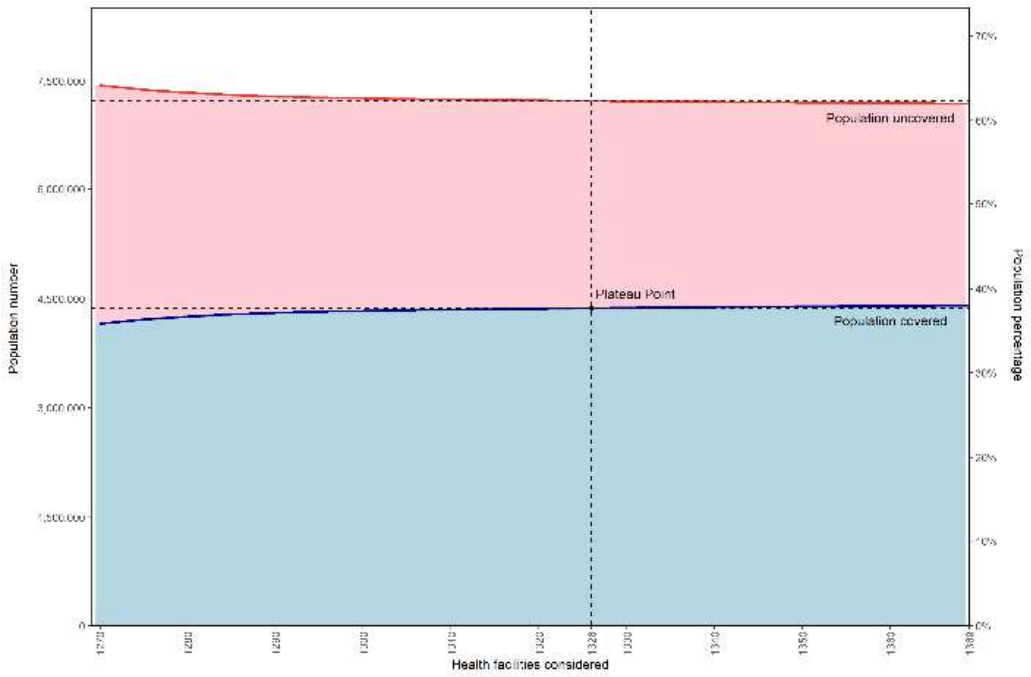
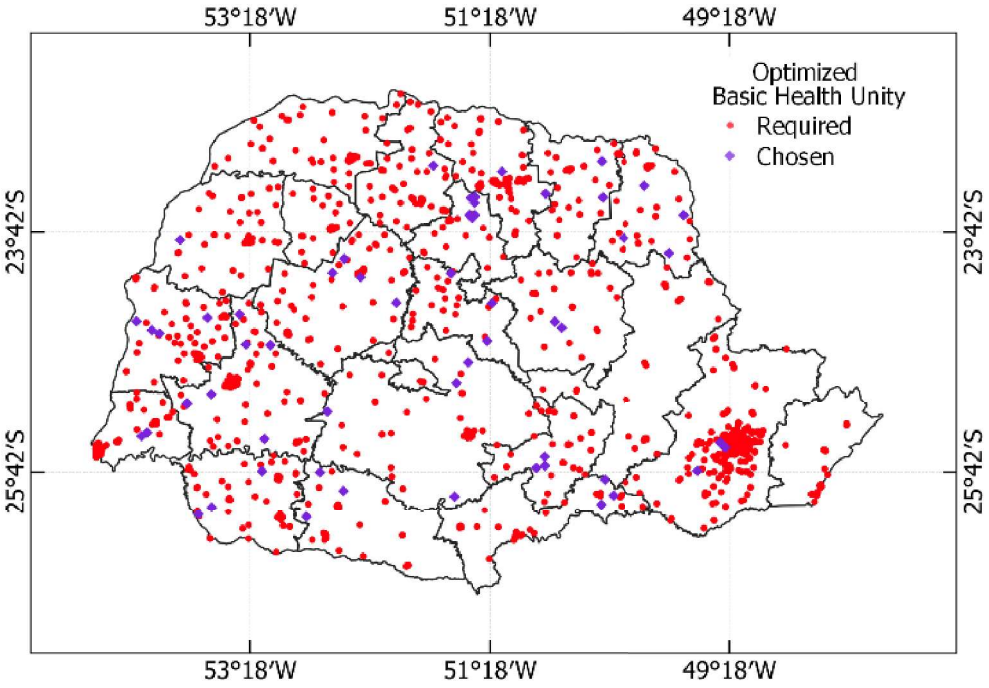


Figure 3. Distribution of required and candidate facilities for COVID-19 vaccination, and the progress of population coverage while adding new BHUs.

Panel A in Figure 4 shows the locations of the optimized chosen facilities, and B shows the Basic Health Units required and chosen overlapping the percentage of people vaccinated in each municipality. Notably some of the new BHUs were addressed to locals when the vaccination was below 70%.

A



B

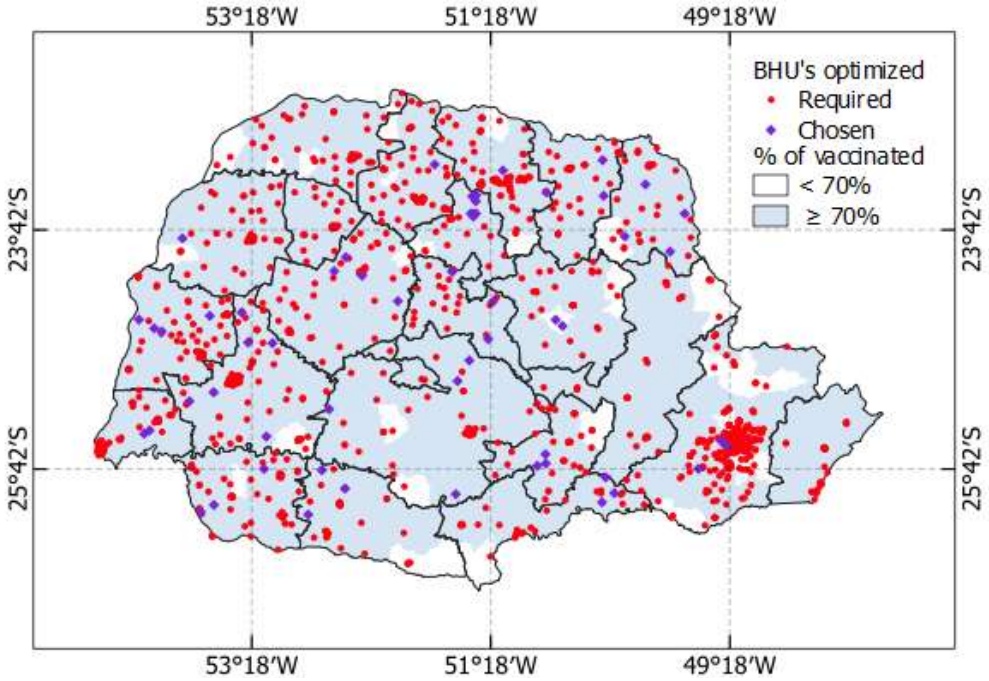


Figure 4. Distribution of the Optimized Basic Health Unity and municipalities vaccination coverage.

256

257

258

Discussion

Measuring vaccination coverage is a keyway to evaluate the effectiveness of a vaccination program and access to healthcare (23). The use of GIS technologies as location-allocation has proved to be an important tool to develop strategies to select new vaccination sites to increase vaccine coverage against COVID-19. In this study, for example, the possible recruitment of 56 new Basic Health Units to help the 1,269 establishments already used for vaccine application would contribute to a coverage gain of 2.15%, which would benefit 250,077 people, that is, almost 38 % of the population of Paraná would have access to the vaccine 7 minutes away by car from the nearest UBS.

Access to vaccines is just one of the four pillars that ensure the immunization of the population. The other three crucial elements are the development and production of vaccines, the acquisition of enough doses, and the distribution of vaccines to the population (5). The race of countries for immunization is fierce, in which the more developed countries have benefited greatly in relation to the low-income countries, which practically depended on donations (24). Therefore, less developed countries are expected to take longer and face more challenges to immunizing their populations (24).

Although vaccination is widely recognized as one of the most effective public health interventions for preventing communicable diseases, saving lives, and reducing disease burden (25), many people are reluctant to receive the immunizer as shown in some studies in which about 91.3 % of the adult population in China accepts the vaccine against COVID-19, 64% in the United Kingdom (25), and globally is around 72% of acceptance (26). Therefore, more important than having access to the vaccine, other strategies are essential to encourage vaccination in the population (26).

Despite of having a history of successful government policies for mass vaccination, including coordinated vaccination campaigns at the country level, effective communication strategies, free availability of doses (27), the vaccination against COVID-19 in Brazil has been a challenging process of immunization. In addition, even with a high number of confirmed cases and deaths from COVID-19, the country's vaccination campaign had a slow start (27) mainly attributed to low vaccine distribution, high hesitancy among the population, fake news, and political issues (28, 29, 30). Thus, a unified effort across all branches of government is crucial in developing a comprehensive policy that effectively addresses the healthcare needs of the population.

A positive contribution to policy maker may come from spatial analysis that can be used to increase vaccination rates by identifying areas with low vaccination coverage, and vaccine hesitancy targeting actions where interventions are needed (31). Another strategy is to use GIS to identify vulnerable populations based on social determinants of health, such as individuals living in poverty or in remote areas, who may have limited access to vaccines (32). Additionally, GIS can be used to identify barriers to vaccination access, and then develop solutions to overcome these barriers (10, 33).

From the point of view of strategic planning for the expansion of vaccine coverage against COVID-19, GIS technology using location-allocation fulfills its role by providing valuable information for health managers to plan the next steps to implement this tool. However, the participation of other government sectors is important to optimize this resource based on the logistical expenses required in the distribution of vaccines to these new locations.

Conclusion

Our work detailed the sociodemographic characteristics of people from Paraná vaccinated against COVID-19 and presented 56 more BHU would cover substantially the largest number of people in the state to expand vaccination coverage.

Acknowledgements

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Weinreich DM, Sivapalasingam S, Norton T, Ali S, Gao H, Bhore R, et al. REGN-COV2, a Neutralizing Antibody Cocktail, in Outpatients with Covid-19. N Engl J Med. 2020.
2. WHO (World Health Organization). Strategic preparedness, readiness and response plan to end the global COVID-19 emergency in 2022. World Health Organization; 2022.
3. Ritchie H, Mathieu E, Rodés-Guirao L, Appel C, Giattino C, Ortiz-Ospina E, et al. Coronavirus pandemic (COVID-19). Published online at OurWorldInData. org. Retrieved from:[Online Resource]. https://ourworldindata. org/coronavirus; 2020.
4. Li Z, Lu J, Lv J. The Inefficient and Unjust Global Distribution of COVID-19 Vaccines: From a Perspective of Critical Global Justice. Inquiry. 2021;58:469580211060992.
5. Wouters OJ, Shadlen KC, Salcher-Konrad M, Pollard AJ, Larson HJ, Teerawattananon Y, et al. Challenges in ensuring global access to COVID-19 vaccines: production, affordability, allocation, and deployment. Lancet. 2021;397(10278):1023-34.
6. Bartsch SM, O'Shea KJ, Ferguson MC, Bottazzi ME, Wedlock PT, Strych U, et al. Vaccine Efficacy Needed for a COVID-19 Coronavirus Vaccine to Prevent or Stop an Epidemic as the Sole Intervention. Am J Prev Med. 2020;59(4):493-503.
7. Utazi CE, Thorley J, Alegana VA, Ferrari MJ, Takahashi S, Metcalf CJE, et al. Mapping vaccination coverage to explore the effects of delivery mechanisms and inform vaccination strategies. Nat Commun. 2019;10(1):1633.
8. Sarker AR, Akram R, Ali N, Chowdhury ZI, Sultana M. Coverage and Determinants of Full Immunization: Vaccination Coverage among Senegalese Children. Medicina (Kaunas). 2019;55(8).
9. Mihigo R, Okeibunor J, Masresha B, Mkanda P, Poy A, Zawaira F, et al. IMMUNIZATION AND VACCINE DEVELOPMENT: Progress towards High and Equitable Immunization Coverage in the Africa Region. J Immunol Sci. 2018;Suppl(1):1-9.
10. Krzysztofowicz S, Osińska-Skotak K. The Use of GIS Technology to Optimize COVID-19 Vaccine Distribution: A Case Study of the City of Warsaw, Poland. Int J Environ Res Public Health. 2021;18(11).
11. Ali D, Levin A, Abdulkarim M, Tijjani U, Ahmed B, Namalam F, et al. A cost-effectiveness analysis of traditional and geographic information system-supported microplanning approaches for routine immunization program management in northern Nigeria. Vaccine. 2020;38(6):1408-15.

12. Ahasan R, Hossain MM. Leveraging GIS and spatial analysis for informed decision-making in COVID-19 pandemic. Health Policy Technol. 2020.
13. Silva LL, de Carvalho Dutra A, de Andrade L, Iora PH, Rodrigues Ramajo GL, Peres Gualda IA, et al. Emergency Care Gap in Brazil: Geographical Accessibility as a Proxy of Response Capacity to Tackle COVID-19. Front Public Health. 2021;9:740284.
14. Kamel Boulos MN, Geraghty EM. Geographical tracking and mapping of coronavirus disease COVID-19/severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) epidemic and associated events around the world: how 21st century GIS technologies are supporting the global fight against outbreaks and epidemics. Int J Health Geogr. 192020. p. 8.
15. Polo G, Acosta CM, Ferreira F, Dias RA. Location-allocation and accessibility models for improving the spatial planning of public health services. PLoS One. 2015;10(3):e0119190.
16. IBGE. Instituto Brasileiro de Geografia e Estatística. Séries estatísticas & séries históricas. Rio de Janeiro2018.
17. Souza EL, Eshriqui I, Masuda ET, Bonfim D, Barra RP, Paresque MAC. Diagnosis of vaccination rooms in Brazilian primary health care centers taking part in the PlanificaSUS project, 2019. Epidemiol Serv Saude. 2022;31(2):e2022069.
18. Tatem AJ. WorldPop, open data for spatial demography. Sci Data. 2017;4:170004.
19. Swanwick RH, Read QD, Guinn SM, Williamson MA, Hondula KL, Elmore AJ. Dasymetric population mapping based on US census data and 30-m gridded estimates of impervious surface. Sci Data. 2022;9(1):523.
20. Birch CPD, Oom SP, Beecham JA. Rectangular and hexagonal grids used for observation, experiment and simulation in ecology. Ecological Modelling. 2007;206(3):347-59.
21. Zhang L, Pfoser D. Using OpenStreetMap point-of-interest data to model urban change-A feasibility study. PLoS One. 2019;14(2):e0212606.
22. Augusto Hernandes Rocha T, Grapiuna de Almeida D, Shankar Kozhumam A, Cristina da Silva N, Bárbara Abreu Fonseca Thomaz E, Christine de Sousa Queiroz R, et al. Microplanning for designing vaccination campaigns in low-resource settings: A geospatial artificial intelligence-based framework. Vaccine. 2021;39(42):6276-82.
23. Cutts FT, Claquin P, Danovaro-Holliday MC, Rhoda DA. Monitoring vaccination coverage: Defining the role of surveys. Vaccine. 2016;34(35):4103-9.
24. Peacocke EF, Heupink LF, Frønsdal K, Dahl EH, Chola L. Global access to COVID-19 vaccines: a scoping review of factors that may influence equitable access for low and middle-income countries. BMJ Open. 2021;11(9):e049505.

392 25. Ali I, Ali S, Iqbal S. COVID-19 Vaccination: Concerns About Its Accessibility,
393 Affordability, and Acceptability. Front Med (Lausanne). 2021;8:647294.
394 26. Kelly BJ, Southwell BG, McCormack LA, Bann CM, MacDonald PDM, Frasier AM,
395 et al. Predictors of willingness to get a COVID-19 vaccine in the U.S. BMC Infect Dis.
396 2021;21(1):338.
397 27. Ferreira LS, Darcie Marquitti FM, Paixão da Silva RL, Borges ME, Ferreira da Costa
398 Gomes M, Cruz OG, et al. Estimating the impact of implementation and timing of the COVID-
399 19 vaccination programme in Brazil: a counterfactual analysis. Lancet Reg Health Am.
400 2023;17:100397.
401 28. The L. COVID-19 in Brazil: "So what?". The Lancet. 2020;395(10235):1461.
402 29. Galhardi CP, Freire NP, Fagundes MCM, Minayo MCS, Cunha I. Fake News and
403 vaccine hesitancy in the COVID-19 pandemic in Brazil. Cien Saude Colet. 2022;27(5):1849-
404 58.
405 30. Lotta G, Fernandez M, Kuhlmann E, Wenham C. COVID-19 vaccination challenge:
406 what have we learned from the Brazilian process? Lancet Glob Health. 2022;10(5):e613-e4.
407 31. Mollalo A, Mohammadi A, Mavaddati S, Kiani B. Spatial Analysis of COVID-19
408 Vaccination: A Scoping Review. Int J Environ Res Public Health. 2021;18(22).
409 32. Qi F, Barragan D, Rodriguez MG, Lu J. Evaluating spatial accessibility to COVID-19
410 vaccine resources in diversely populated counties in the United States. Front Public Health.
411 2022;10:895538.
412 33. Bauer C, Zhang K, Lee M, Jones M, Rodriguez A, de la Cerda I, et al. Real-time
413 geospatial analysis identifies gaps in COVID-19 vaccination in a minority population. Sci Rep.
414 2021;11(1):18117.

415

Artigo 3: Observational study to evaluate spatial distribution of COVID-19 cases associated to Social Determinants of Health disparities amongst communities in North Carolina, United States

Observational study to evaluate spatial distribution of COVID-19 cases associated to Social Determinants of Health disparities amongst communities in North Carolina, United States

Authors

Lincoln Luis Silva[1,2], Justine Seidenfeld[2], Anna Tupetz[2], Alexander Limkakeng[2], Cassandra Fiorino[3], Catherine Staton[2], John Purakal[2], Rosilene Fressatti Cardoso[1,†], Joao Ricardo Nickenig Vissoci[2,4,†]

Affiliation

[1] Post-Graduation Program in Biosciences and Physiopathology, State University of Maringá, Paraná, Brazil;
[2] Division of Emergency Medicine, Department of Surgery, Duke University School of Medicine, Durham, NC, United States of America;
[3] Duke University School of Medicine, Durham, NC, United States of America;
[4] Duke Global Health Institute, Duke University, Durham, NC, United States of America.

† These authors have contributed equally to this work

Corresponding author

Professor Dr. Joao Vissoci jnv4@duke.edu

Abstract

Background: In the United States, minorities are disproportionately infected, hospitalized, and are dying at high rates during the COVID-19 pandemic. Further, there are many relevant Social Determinants of Health (SDOH) that could impact the pandemic, including employment, urban crowding, multigenerational households, poor health literacy, and inadequate access to health care or food. Therefore, mapping regions with deprived SDOH could lead to development of public health strategies to mitigate the number of cases in these populations.

Methods: We analyzed the geospatial associations of COVID-19 infections and SDOH to identify areas of overlap. Our sample comprised all patients in a North Carolina healthcare system's registry who tested positive for COVID-19 from March 2020 to February 2021 from six counties in North Carolina. Patients' addresses were geo-referenced and analyzed by Kernel Density Estimation to identify population-dense outbreaks of COVID-19 (hotspots). A set of 14 SDOH variables for each county were collected from the American Community Survey (ACS-1 and 5) and the Economic Research Service. Principal Component Analysis was applied to SDOH variables in order to reduce dimensions down to 3 geographical SDOH categories: Protective SDOH, High-Risk SDOH and Increased Vulnerability for Infection. Using Multivariate Clustering Analysis (MCA), three clusters of census tracts were categorized according to SDOH indicators: decreased social disparities (DSD), equivocal social disparities (ESD) and increased social disparities (ISD). Kruskal-Wallis and Dunn's Post-Hoc adjusted with Bonferroni were utilized to verify any difference in the proportion of patients residing in the different clusters at significance $p < 0.05$.

Results: A total of 13,733 patients were included in the study. The patients predominantly reside in Durham County (55.4%), are women (56.96%), and between 40 and 69 years old (41.9%). Further, patients are predominantly white (38.7%), non-Hispanic (79.63%), and single (49.6%). The concomitant analysis of KDE and MCA showed an overlap of COVID-19 hotspots with areas of ISD. The MCA revealed that of 308 census tracts constituted by six counties, 40 form ISD clusters (vs. 109 ESD; vs. 159 DSD). In addition, ISD clusters have the highest rates of infection, with 179.8 patients per 10,000 (vs. 81.7 ESD; vs. 60.1 DSD). The ISD cluster was the most densely populated and was significantly more densely populated from the ESD and DSD clusters ($p < 0.05$).

Conclusion: In this sampling of COVID-19 patients, a disproportionate number of patients come from areas with increased social disparities, suggesting that a health policy will need to be directed towards addressing negative and vulnerability SDOH to curtail pandemic-related outbreaks.

Keyword: COVID-19; spatial analysis; epidemiology; social determinants of health; social vulnerability.

Introduction

COVID-19 was declared as a pandemic on March 11, 2020 and since then it has challenged the United States (US) Health System (1). As of December 2021, the US is the country with the highest number of COVID-19 cases worldwide, with more than 54 million confirmed cases and 809 thousand deaths (2). Infection with SARS-CoV-2 , is underreported because many people are asymptomatic or mild, and therefore do not prompt care seeking, nor testing (3). This elevated number of cases and deaths reveals the systemic frailties of US healthcare system (4), and the flawed response to the pandemic fundamentally aggravated by inattention to the Social Determinants of Health (SDOH) (5).

The COVID-19 pandemic has demonstrated that SDOH are profoundly linked to COVID-19 morbidity and mortality (6). SDOH are the conditions in which people are born, grow, live, work, and age. These circumstances are shaped by the distribution of money, power, and resources at global, national, and local levels (7). Many SDOH including unemployment, decreased access to care or food, unstable housing, and multigenerational homes have shown a disproportionate effect on COVID-19 spread and outcomes (6). Further, there is mounting evidence that minority race and ethnicity are similarly linked to disproportionate disease burden (8). However, it has not been determined whether these factors are also related to the spatiality for the distribution of COVID-19 cases.

Geographic information systems (GIS) analysis on the medical field increases the ability to monitor diseases and identify their causes (9, 10). In addition, GIS allows policy makers to easily visualize problems in relation to existing health and social services and the natural environment, thus allowing more effective targeting of resources (11). Furthermore, GIS is an ideal platform for the convergence of disease-specific information and their analyses in relation to population settlements, surrounding social and health services and the natural environment (12).

Additionally, studies of SDOH are widely applied in geography, economics, and public health to identify inequalities and sub-populations at risk to a determined event (13). However, COVID-19 studies have not presented the main role of social vulnerability measurements along with sociodemographic elements of place to explain patterns of COVID-19 cases within local level (13). These studies mainly focused on the number of cases, as for example between September 2020 to January 2021, North Carolina (NC) presented clusters of high incidences of COVID-19 between counties across the state (14). This region is subject of interest to verify the impact of SDOH on the population because NC is the ninth most populous state in the US, and while the majority of the NC population is White, the state ranks sixth and eleventh among all US states in Black and Latins population size, respectively (15).

Therefore, since the social determinants of health are crucial elements to consider in evaluating the spatial and temporal dynamics of the COVID-19 pandemic (13), the objective of this study was to conduct a spatial analyses of county-level SDOH and regional COVID-19 infection outbreaks to demonstrate the association between SDOH, and sociodemographic variables on COVID-19 distribution in a region of North Carolina based on data of patient-level retrieved from a large health facility from March 2020 to February 2021.

Methods

Settings

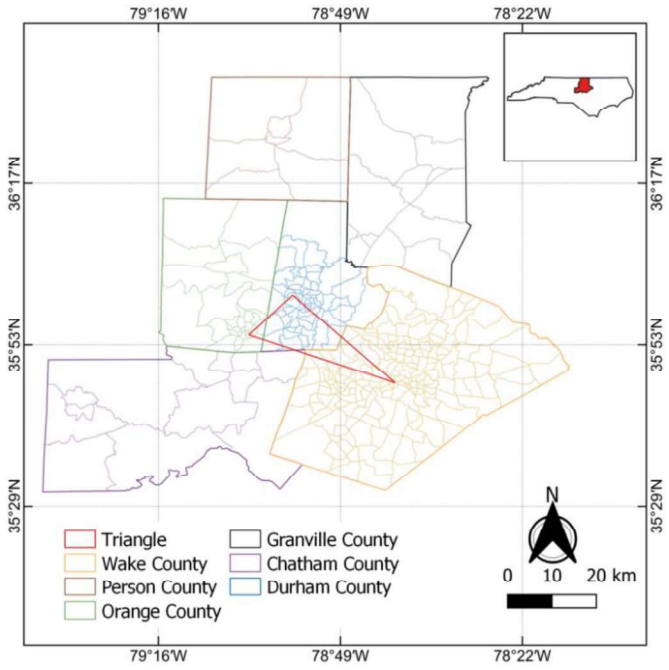
Study design

This is an observational, cross-sectional, and ecological study based on patient-level data of individuals diagnosed with COVID-19 from February 2020 to March 2021, analyzed with geospatial approaches to assess the relation between social determinants of health and the distribution of COVID-19 cases in the North Carolina`s census-tract limits.

Participants

According to the preliminary US Census Tract of 2019 (https://www.census.gov), it is estimated that these six counties have a total of 1,695,774 inhabitants distributed into 308 census-tracts (Figure 1). The area is part of Research Triangle Park because of the three major research universities that occupy three cities that include: Durham, home to Duke University, Chapel Hill, home to University of North Carolina, and Raleigh with North Carolina State

553 University (16, 17). The area has expanded into a nucleus of intellectual activity in
554 environmental health sciences in the last decades (18).
555



556
557 Figure 1. Region of interest in this study composed of six counties: Wake, Person, Orange,
558 Granville, Chatham and Durham. The Triangle is a representation of a technological region.
559

560 Data Analysis

561 Data sources and selected variables

562 A set of 14 SDOH variables of each census-tract for each county were collected from
563 the American Community Survey 1 and 5-Year Data (2019)
564 (https://www.census.gov/data/developers/data-sets/ACS-supplemental-data.html) and the
565 Economic Research Service (https://www.ers.usda.gov/data-products/food-environment-
566 atlas/data-access-and-documentation-downloads/#Current%20Version). Table 1 describes the
567 variables selection. Additionally, the shapefiles that contain the territorial limits of US, NC,
568 counties and census-tract were extracted from the US Census Bureau
569 (https://www.census.gov/geographies/mapping-files/time-series/geo/carto-boundary-
570 file.html).

571 We collected restricted and protected data on the 13,733 patients from Duke Health with
572 a positive polymerase chain reaction or rapid test for COVID-19 using Electronic Medical
573 Record. The patients selected came from the referred counties of 1) Durham, 2) Wake, 3)

574 Granville, 4) Orange, 5) Person, and 6) Chatham, attended at Duke's Health Facility in Durham
575 were included in this study.

Table 1. Description and source of social determinants of health variables selected for the study.

Variable	Description	Source
Percentage of people below poverty	Income in the past 12 months (in 2019 inflation-adjusted dollars)	U.S Census
Unemployment	Unemployment Rate	U.S Census
Number of people uninsured	Number of people without health insurance	U.S Census
Percentage of houses without vehicle	Physical housing characteristics for occupied housing units	U.S Census
Household size	Average household size	U.S Census
Percentage of people with limited English per household	Limited English-speaking households	U.S Census
Total of health professionals	Healthcare practitioners and technical occupations	U.S Census
Median income	Income in the past 12 months (in 2019 inflation-adjusted dollars)	U.S Census
Number of people above 16 years-old with high school degree	Occupation by sex for the civilian employed population 16 years and over	U.S Census
Number of people insured	Number of people with health insurance	U.S Census
Percentage of houses ≥ 1 vehicle(s)	Percentage of houses with 1 or more vehicles	U.S Census
Employment	Employment rate	U.S Census
Number of total populations	Number of total populations, whites, blacks and Hispanics	U.S Census
Food desert areas	Low income and low access tract using vehicle access or low income and low access tract measured at 20 miles	Economic Research Service

Low access to healthy food	Low-income population count beyond 1/2 mile for urban areas or 10 miles for rural areas from supermarket	Economic Research Service

576
577
578 Statistical analysis

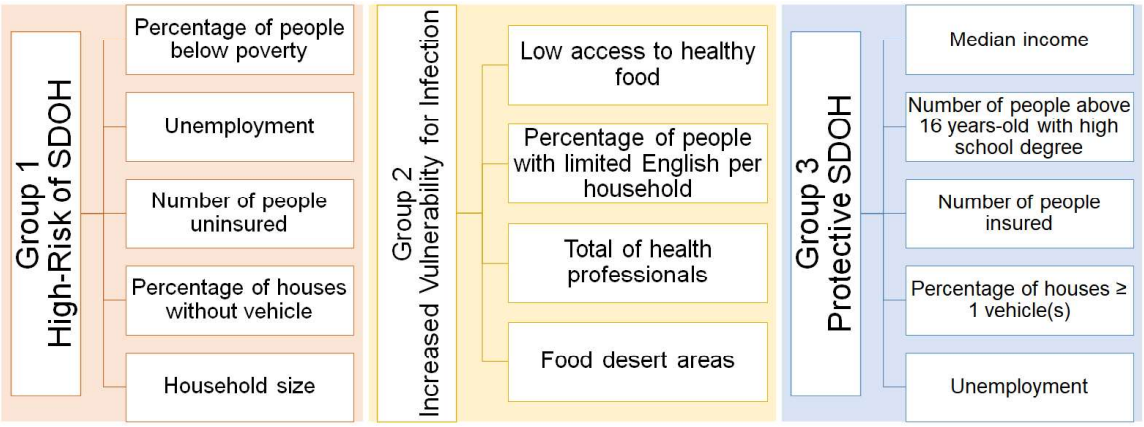
579 To identify the regions presenting an association between SDOH and geospatial
580 clustering, we proceeded within four steps. The first step was dedicated to verifying areas
581 densely populated by patients with COVID-19 using a hotspot approach. Second, Principal
582 Component Analysis (PCA) was applied to reduce the number of variables into few dimensions
583 without losing information, in order to simplify the complexity data and analyzing all variables
584 at once instead of separately. Third, Multivariate Clustering analysis was performed to find
585 where all variables within the same group of variables from the PCA within each census-tract
586 are like each other and capable to create a cluster. Four, Kruskall-Wallis was applied to verify
587 whether the patients in different type of cluster is statistically different from each other in terms
588 of proportion of patients. With these steps is possible to verify the association between SDOH
589 and spatial distribution of COVID-19 cases. All the statistical analysis was performed using R
590 Studio version 2021.09.01 and ArcGis Pro 2.8.3.
591
592 Descriptive analysis

593 The information of the following variables: gender, age, race, ethnicity, and marital
594 status of all patients included in the study was presented using absolute and relative frequency
595 in each county.
596
597 Hotspots

598 All 13,733 patient's addresses were georeferenced and analyzed by Kernel Density
599 Estimation (KDE) to identify population-dense outbreaks of COVID-19 (hotspots) in each
600 county. KDE is a spatial technique that weights the distribution of patient's residency according
601 to their proximity to each other, making possible for researchers to verify whether patients are
602 mostly likely sparsely distributed dispersed, or clustered (19).
603
604
605

606 Principal Component Analysis

607 In other to retain as much as possible of the data's variation provided from SDOH
608 variables to be evaluated in spatial analysis, and at the same time, minimizing information loss
609 (20, 21), Principal Component Analysis (PCA) was performed using oblique rotation for three
610 group of variables. The first group was called "High-Risk of SDOH", in which contains the
611 following variables: percentage of people below poverty, unemployment, number of people
612 uninsured, percentage of houses without vehicle, and household size; the second group as
613 "Increased Vulnerability for Infection" with the following variables: low access to healthy
614 food, percentage of people with limited English per household, total of health professionals,
615 and food desert areas variables; and a third group as "Protective SDOH" with median income,
616 number of people above 16 years-old with high school degree, number of people insured,
617 percentage of houses ≥ 1 vehicle(s), and employment.



618

619 Figure 2. Structure of variables in each group performed in principal component analysis.

620

621 Multivariate Clustering Analysis

622 Multivariate analysis is used for analyzing data with more than one variable under
623 consideration. In addition, the clustering approach establishes a set of meaningful groups of
624 similar objects by investigating relationships between objects using an algorithm that looks up
625 for variables that are similar, putting them together in a cluster or segment, and separating them
626 from other. Therefore, to assess the formation of clusters between census-tracts, a multivariate
627 clustering technique using K Means algorithm was applied on the three group of variables
628 "High-Risk of SDOH", "Increased Vulnerability for Infection", and "Protective SDOH" (22).

629

630

631

632 Evaluation of the disproportionate number of patients in the clusters

633 In order to verify if the number of patients is related to a certain cluster, a Kruskal–
634 Wallis analysis followed by Dunn's Multiple Comparison Test post hoc adjusted by Bonferroni
635 was performed. The proportion of patients between each cluster was compared and considered
636 statistically significant when $p < 0.05$.
637
638 Ethics
639 Disclosure
640 All patient-level information used in this study was approved by Duke's University
641 Institutional Review Board (IRB) number xxx/2020.
642
643 Data availability
644 All information regarding patients' sociodemographic and clinical characteristics is
645 protected under Duke's University IRB. However, the US Census Tract datasets for this study
646 can be found in the Figshare repository: http://doi.org/.
647
648 **Results**
649
650 Population information
651 The descriptive analysis of the study population is shown in Table 2. The patients
652 predominantly reside in Durham County (55.41%), are women (56.96%), and between 40 and
653 69 years old (41.86%). Further, patients are predominantly white (38.67%), from other than
654 Hispanics (79.63%), and single (49.60%).
655
656
657
658
659
660
661
662

Table 2. Descriptive information about the 13,733 patients in six counties in North Carolina in 2021.

Characteristics	Counties						
	All	Chatham	Durham	Granville	Orange	Person	Wake
	N (%)						
Patients							
Number	13,733 (100)	126 (0.92)	7,609 (55.41)	840 (6.12)	845 (6.15)	481 (3.50)	3,832 (27.90)
Gender							
Male	5,910 (43.04)	61 (48.41)	3,295 (43.30)	360 (42.86)	375 (44.38)	181 (37.63)	1,638 (42.75)
Female	7,823 (56.96)	65 (51.59)	4,314 (56.70)	480 (57.14)	470 (55.62)	300 (62.37)	2,194 (57.25)
Age (years old)							
< 18	1,727 (12.58)	6 (4.77)	1,248 (16.40)	94 (11.19)	86 (10.18)	68 (14.14)	225 (5.87)
18 – 39	4,740 (34.52)	34 (26.98)	2,702 (35.51)	219 (26.07)	259 (30.65)	130 (27.03)	1,396 (36.43)
40 – 69	5,748 (41.86)	58 (46.03)	2,908 (38.22)	410 (48.81)	376 (44.50)	212 (44.07)	1,784 (46.56)
> 70	1,518 (11.04)	28 (22.22)	751 (9.87)	117 (13.93)	124 (14.67)	71 (14.76)	427 (11.14)
Race							
Blacks	5,047 (36.75)	21 (16.67)	3,279 (43.09)	268 (31.90)	123 (14.56)	163 (33.89)	1,193 (31.13)
Whites	5,310 (38.67)	85 (67.46)	2,064 (27.13)	457 (54.41)	565 (66.86)	299 (62.16)	1,840 (48.02)
Others	3,376 (24.58)	20 (15.87)	2,266 (29.78)	115 (13.69)	157 (18.58)	19 (3.95)	799 (20.85)
Ethnicity							
Hispanics	2,798 (20.37)	12 (9.52)	2,086 (27.41)	87 (10.36)	121 (14.32)	12 (2.49)	480 (12.53)

Others	10,935	114	5,523	753	724	469	3,352
	(79.63)	(90.48)	(72.59)	(89.64)	(85.68)	(97.51)	(87.47)
Marital status							
Single	6,811	37	4,251	322	332	199	1,670
	(49.60)	(29.37)	(55.87)	(38.33)	(39.29)	(41.37)	(43.58)
Married	4,937	71	2,288	374	403	203	1,598
	(35.95)	(56.35)	(30.07)	(44.52)	(47.69)	(42.20)	(41.70)
Others	1,985	18	1,070	144	110	89	564
	(14.45)	(14.29)	(14.06)	(17.14)	(13.02)	(18.50)	(14.72)

Kernel Density Estimation (KDE)

Figure 3 shows the hotspot regions in red according to the concentration of patients in a particular area. The KDE technique was performed in each county independently in order to avoid a concentration of all hotspot areas in Durham County because it's the county with the highest number of patients. Therefore, the size of hotspots is different in each county. In addition, under the hotspots layer was also plotted the information about SDOH variables. Analyzing the stacked layers is possible to observe an overlapping pattern amongst the hotspots and the areas with high percentages of people below poverty, number of people uninsured, houses with no vehicle, household size, low access to healthy food, households with limited English, and food desert areas. On the other hand, we found hotspots overlapping regions with low rates of median income, and few numbers of health workers.

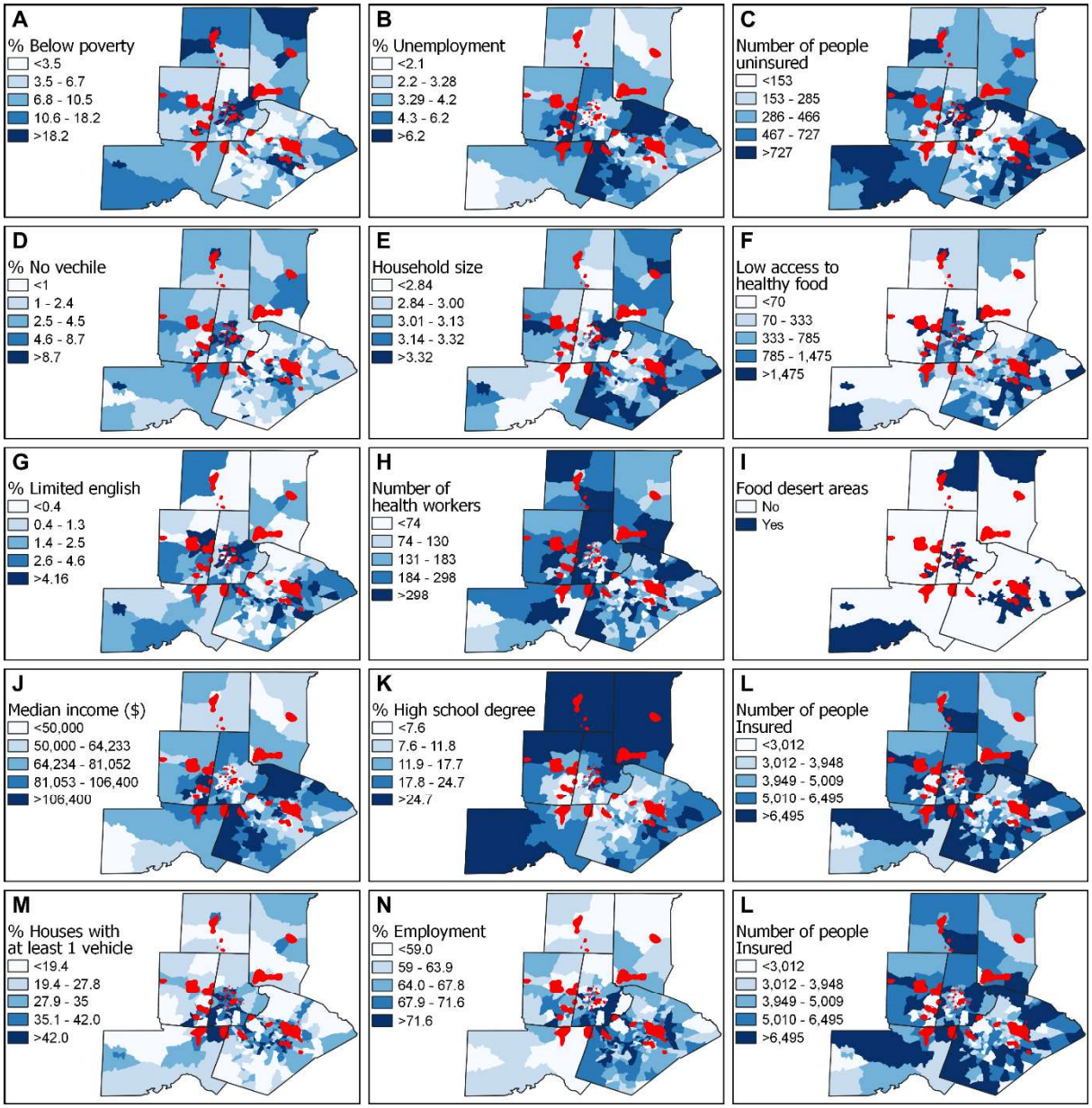


Figure 3. Hotspot areas and descriptive distribution of social determinants of health variables between some counties in North Carolina.

Multivariate clustering

After performing the PCA analysis with 3 groups of variables (PCA scores can be accessed in the supplemented material), the multivariate clustering was applied and it was verified that three clusters were identified and named as: “increased”, “equivocal”, and “decreased” social challenges. Cluster of increased social challenges is represented by strong scores of high-risk SDOH and Increased Vulnerability for Infection groups and weakened scores of protective SDOH groups . Decreased social challenges cluster on the other hand, is represented by strong scores of protective SDOH groups and weakened scores of high-risk

SDOH and Increased Vulnerability for Infection groups. Equivocal clusters share strengths and weaknesses scores of the three groups. Figure 4 presents the respective clusters and the hotspots overlapping these regions.

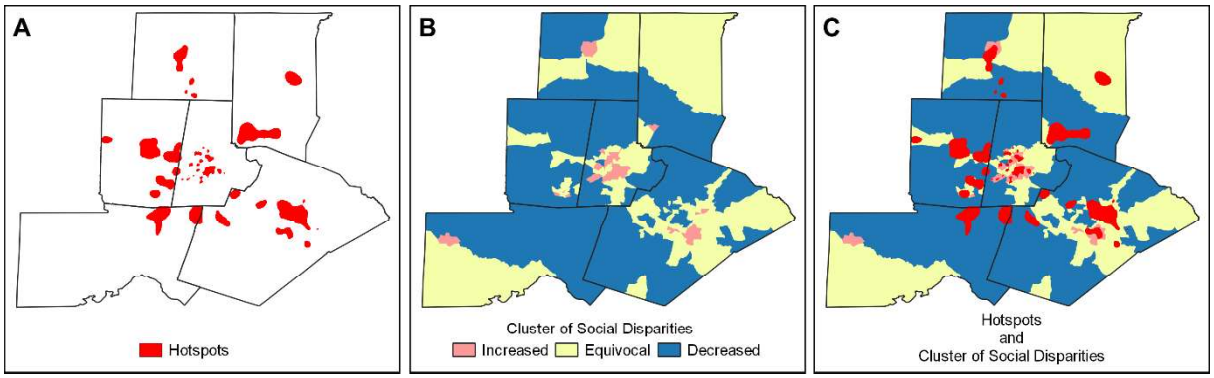


Figure 4. In A shows the hotspots. In B shows the regions with the respective cluster. In C is the hotspots overlapping clusters.

Proportion analysis of patients in each cluster

Of 308 census-tract, 40 were identified as clusters of increased social challenge, 109 equivocal, and 159 decreased. In Table 3 is shown the number of patients in each cluster. In terms of numbers, most patients belong to areas with decreased social challenges clusters (39.85%). However, those who are in the increased social challenges clusters are in Durham County (38.80%).

Table 3. Descriptive analysis of patients in each cluster created in the multivariate clustering

Clusters of social challenges	Counties						
	All	Chatham	Durham	Granville	Orange	Person	Wake
	Number of patients (%)						
Increased[a]	3,382 (24.63)	2 (1.59)	2,952 (38.80)	50 (5.95)	18 (2.13)	62 (12.89)	298 (24.63)
Equivocal[b]	4,879 (35.52)	110 (11.11)	2,559 (33.63)	378 (45.00)	243 (28.76)	166 (34.51)	1,519 (35.53)

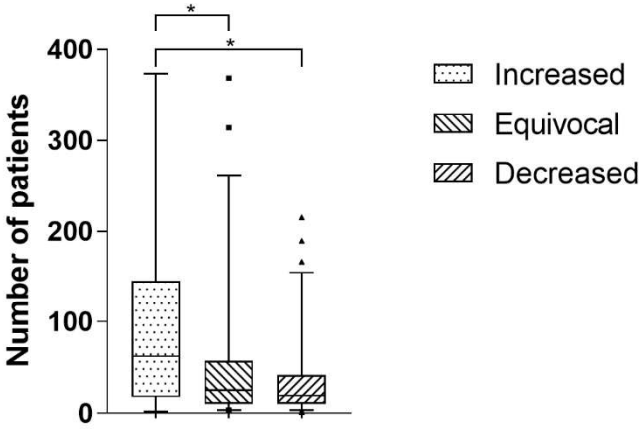
Decreased[b]	5,472 (39.85)	14 (87.30)	2,098 (27.57)	412 (49.05)	584 (69.11)	253 (52.60)	2,015 (39.84)

[a]cluster that the proportion of patient is different ($p < 0.05$);

[b]cluster that the proportion of patient is not different ($p > 0.05$).

705

706 The Kruskal-Wallis Test showed that there is a difference between the groups ($p < 0.05$),
707 and Dunn's post-Hoc showed that the "Increased" group is statistically different from the
708 "Equivocal" and "Decreased" groups ($p < 0.05$). In other words, considering 10,000 inhabitants
709 per census-tract, 179.81 patients are in increased clusters, 81.72 in equivocal, and 60.08 in
710 decreased, meaning that a disproportionate number of patients come from areas with increased
711 social challenges. The Figure 5 represents the number of patients in each cluster per census-
712 tract.



713

714 Figure 5. Graphical representation of the sum of number of patients in each cluster. * Indicates
715 that the difference group is statistically significant different from the other group ($p < 0.05$).

716

717

718 **Discussion**

719

720 This study verified the spatial distribution of COVID-19 cases related to local disparities
721 of Social Determinants of Health. Here we presented regions most densely affected by COVID-
722 19 in a set of counties in North Carolina. It was verified that the SDOH are related to the
723 distribution of cases in the region, where the proportion of patients living in clusters of increased
724 social challenges is significantly higher in comparison to equivocal and decreased clusters.

Therefore, the US depreciable gave inattention to the social determinants of health during the pandemic times.

The US preparedness has been evident, especially in ethnic minority and underserved populations (23). Additionally, the absence of investment in the health system created an opportunity that made it easier for COVID-19 to spread unevenly quickly across the US population (6). Notably worth to mention that socioeconomic status and accessibility to care are ultimately involved in the number of COVID-19 cases (6, 24).

The impact of COVID-19 has been seen across all socioeconomic status, however it deeply affects those in the lower socioeconomic categories (25), in which people earning less is associated with devastating consequences of post-COVID-19 health conditions compared to their socioeconomically better off counterparts, while the poor might be more likely to suffer or die during the outbreak (26). Here we also noticed hotspots overlapping regions with low income, elevated rates of uninsured, and unemployment. To further the concern on low income, a lot of US people lost their jobs because of COVID-19 restrictions, and as result, the unemployment rate reached a peak in April 2020 at 14.7% nationwide, an unprecedented joblessness amount (27). Although the negative consequences of unemployment do not affect all individuals in the same way (28), different studies have shown how unemployment affects COVID-19 mortality and morbidity (29, 30, 31). Therefore, this is a topic worthy of attention to perform a counter measure in a short-term resolution.

The access to care is not equally distributed across the US (32). Some factors also may be involved with the disparities of access to care, such as racism, mistrust, and language barriers (33). People with COVID-19 and poor access to healthcare may delay or are not even tested for the disease and may seek for medical assistance only in complicated stages, resulting in poorer outcomes (34). Last, nearly 1 in 4 newly unemployed workers are likely to lose health coverage during the pandemic (35), and will face significant barriers in accessing care since they have no established provider-patient relationship or health care network (34).

Household size was an important factor taken into consideration in this study because it's expected to have more cases in overcrowded places. In this study we found several hotspots overlapping regions with larger household sizes. Larger households may have a large amount of members who are essential workers, and evidence indicates that those living in neighborhoods with high proportions of essential workers had higher rates of COVID-19 infection and death than those living in other neighborhoods (36).

The advantages of observational studies rely on their relatively low complexity, cost, and ethical constraints compared to randomized trials or other forms of clinical

experimentation. However, we found some limitations in this study that is worth to mention. First, the number of cases in the study does not cover all the cases in the region since we were only able to identify those patients who were attended at the Hospital. Second, the patients did not have the same sociodemographic information used in the census, therefore, we could not evaluate if the living conditions also reflects the spread of COVID-19. Therefore, there may be patients with good social status residing in places with poor rates of social determinants of health, and vice-versa. Yet, our study also has interesting strengths because our data presents information of patient-level, being possible to identify the exact location of a case, and county-level census information helped to make the analysis less biased because we evaluated the distribution of cases at the macroscopic level rather than punctuality where discrepancies could be even greater.

Conclusion

In conclusion, COVID-19 is disproportionally affecting the minorities and vulnerable populations in the US. In addition, the spread of this disease in areas densely populated and limited mitigation capacity due to poor access to high quality of public health or medical care has endorsed the problem. Lastly, the effects of the pandemic on this population will follow them for countless time due to the global economic downturn, the problematic US health care system, and the slow process of society restructuration to be able to insert the minorities into the socioeconomic activity.

Acknowledgement

LLS would like to thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

Funding

Dr. Purakal, of Duke University - Division of Emergency Medicine, was awarded with a grant from Emergency Medicine Foundation for his project titled "Social Determinants of Health and COVID-19 Infection in North Carolina: A Geospatial and Qualitative Analysis" number xxx/2020.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Iuliano AD, Chang HH, Patel NN, Threlkel R, Kniss K, Reich J, et al. Estimating under-recognized COVID-19 deaths, United States, march 2020-may 2021 using an excess mortality modelling approach. Lancet Reg Health Am. 2021;1:100019.
2. Ritchie H, Mathieu E, Rodés-Guirao L, Appel C, Giattino C, Ortiz-Ospina E, et al. Coronavirus pandemic (COVID-19). Published online at OurWorldInData. org. Retrieved from:[Online Resource]. https://ourworldindata. org/coronavirus; 2020.
3. Bai Y, Yao L, Wei T, Tian F, Jin DY, Chen L, et al. Presumed Asymptomatic Carrier Transmission of COVID-19. Jama. 2020;323(14):1406-7.
4. Galvani AP, Parpia AS, Pandey A, Zimmer C, Kahn JG, Fitzpatrick MC. The imperative for universal healthcare to curtail the COVID-19 outbreak in the USA. EClinicalMedicine. 2020;23:100380.
5. Moghadas SM, Galvani AP. The Unrecognized Death Toll of COVID-19 in the United States. Lancet Reg Health Am. 2021;1:100033.
6. Abrams EM, Szefler SJ. COVID-19 and the impact of social determinants of health. Lancet Respir Med. 2020;8(7):659-61.
7. Commission on Social Determinants of H. Closing the gap in a generation: health equity through action on the social determinants of health: final report of the commission on social determinants of health: World Health Organization; 2008.
8. Phiri P, Delanerolle G, Al-Sudani A, Rathod S. COVID-19 and Black, Asian, and Minority Ethnic Communities: A Complex Relationship Without Just Cause. JMIR Public Health Surveill. 2021;7(2):e22581.
9. Longbottom J, Shearer FM, Devine M, Alcoba G, Chappuis F, Weiss DJ, et al. Vulnerability to snakebite envenoming: a global mapping of hotspots. Lancet. 2018;392(10148):673-84.
10. Mullner RM, Chung K, Croke KG, Mensah EK. Geographic information systems in public health and medicine. J Med Syst. 2004;28(3):215-21.
11. Taylor DM, Yeager VA, Ouimet C, Menachemi N. Using GIS for administrative decision-making in a local public health setting. Public Health Rep. 2012;127(3):347-53.
12. Yang B. GIS crime mapping to support evidence-based solutions provided by community-based organizations. Sustainability. 2019;11(18):4889.

13. Jackson SL, Derakhshan S, Blackwood L, Lee L, Huang Q, Habets M, et al. Spatial Disparities of COVID-19 Cases and Fatalities in United States Counties. Int J Environ Res Public Health. 2021;18(16).

14. Andrews MR, Tamura K, Best JN, Ceasar JN, Batey KG, Kearse TA, et al. Spatial Clustering of County-Level COVID-19 Rates in the U.S. International Journal of Environmental Research and Public Health [Internet]. 2021; 18(22).

15. Brandt K, Goel V, Keeler C, Bell GJ, Aiello AE, Corbie-Smith G, et al. SARS-CoV-2 testing in North Carolina: Racial, ethnic, and geographic disparities. Health Place. 2021;69:102576.

16. Nawaz W, Koç M. Case Study: United States of America. Industry, University and Government Partnerships for the Sustainable Development of Knowledge-Based Society: Drivers, Models and Examples in US, Norway, Singapore and Qatar. 2020:9-33.

17. Forbes [Internet]. [cited 2023 Jan 26]. Available from: https://www.forbes.com/sites/davidkroll/2012/10/22/rtp-research-triangle-primer/?sh=1f7ece406a0b.

18. Real AIM. RTP Leaders Unite to Advance Environmental Health. Environmental Health Perspectives. 2006;114(9).

19. King TL, Bentley RJ, Thornton LE, Kavanagh AM. Using kernel density estimation to understand the influence of neighbourhood destinations on BMI. BMJ Open. 2016;6(2):e008878.

20. Jolliffe IT, Cadima J. Principal component analysis: a review and recent developments. Philos Trans A Math Phys Eng Sci. 2016;374(2065):20150202.

21. Groth D, Hartmann S, Klie S, Selbig J. Principal components analysis. Methods Mol Biol. 2013;930:527-47.

22. Steinley D. K-means clustering: A half-century synthesis. British Journal of Mathematical and Statistical Psychology. 2006;59(1):1-34.

23. Cuellar NG, Cuellar MJ, McDiarmid A, Bautista N, Crespo-Fierro M, Infante G, et al. Social Determinants of Health and COVID-19 Behaviors and Beliefs Toward Immunizations Among Latinxs. Hisp Health Care Int. 2021;19(4):221-9.

24. Dalsania AK, Fastiggi MJ, Kahlam A, Shah R, Patel K, Shiau S, et al. The Relationship Between Social Determinants of Health and Racial Disparities in COVID-19 Mortality. J Racial Ethn Health Disparities. 2022;9(1):288-95.

25. Hyder AA, Hyder MA, Nasir K, Ndebele P. Inequitable COVID-19 vaccine distribution and its effects. Bull World Health Organ. 99. Switzerland2021. p. 406-a.

26. Takian A, Kiani MM, Khanjankhani K. COVID-19 and the need to prioritize health equity and social determinants of health. Int J Public Health. 65. Switzerland2020. p. 521-3.
27. Antipova A. Analysis of the COVID-19 impacts on employment and unemployment across the multi-dimensional social disadvantaged areas. Soc Sci Humanit Open. 2021;4(1):100224.
28. Bocchino A, Gilart E, Roman IC, Lepiani I. Unemployment Syndrome during COVID-19: A Comparison of Three Population Groups. International Journal of Environmental Research and Public Health [Internet]. 2021; 18(14).
29. Parolin Z. Unemployment and child health during COVID-19 in the USA. Lancet Public Health. 2020;5(10):e521-e2.
30. Ksinan Jiskrova G, Bobák M, Pikhart H, Ksinan AJ. Job loss and lower healthcare utilisation due to COVID-19 among older adults across 27 European countries. Journal of Epidemiology and Community Health. 2021;75(11):1078.
31. Nghiem N, Wilson N. Potential impact of COVID-19 related unemployment on increased cardiovascular disease in a high-income country: Modeling health loss, cost and equity. PLOS ONE. 2021;16(5):e0246053.
32. Gray DM, 2nd, Anyane-Yeboa A, Balzora S, Issaka RB, May FP. COVID-19 and the other pandemic: populations made vulnerable by systemic inequity. Nat Rev Gastroenterol Hepatol. 2020;17(9):520-2.
33. Warren RC, Forrow L, Hodge DA, Sr., Truog RD. Trustworthiness before Trust - Covid-19 Vaccine Trials and the Black Community. N Engl J Med. 2020;383(22):e121.
34. Shadmi E, Chen Y, Dourado I, Faran-Perach I, Furler J, Hangoma P, et al. Health equity and COVID-19: global perspectives. Int J Equity Health. 19. England2020. p. 104.
35. Woolhandler S, Himmelstein DU. Intersecting U.S. Epidemics: COVID-19 and Lack of Health Insurance. Ann Intern Med. 173. United States2020. p. 63-4.
36. Liu P, McQuarrie L, Song Y, Colijn C. Modelling the impact of household size distribution on the transmission dynamics of COVID-19. J R Soc Interface. 2021;18(177):20210036.

CAPÍTULO III

CONCLUSÕES

O estudo realizado a cerca do preparo do Sistema Único de Saúde frente a COVID-19 mostrou que:

1) Os leitos de unidade de terapia intensiva, ventiladores, e profissionais de saúde não distribuídos de maneira em que a população possa ter um acesso igualitário a esses recursos;
2) A implementação de novos leitos de UTI destinados para atender somente pacientes com COVID-19 não foram destinados aos locais mais necessitados;
3) Regiões norte e nordeste foram as mais afetadas pela COVID-19 pois já estavam despreparadas antes da pandemia.

Em relação alocação de novas Unidas Básicas de Saúde para vacinas no Paraná, verificamos que:

1) O estado do Paraná se organizou de forma com que a população fosse rapidamente vacinadas conforme houvesse a disponibilidade de vacina, o que demonstra o bom preparo do SUS na região;
2) Ainda que tenha tido êxito durante a campanha de vacinação, seria possível melhorar ainda mais com a implementação de 56 novas UBS para que a população atingisse a recomendação de 70% dos vacinados em menor tempo;

Por último, o trabalho desenvolvido nos EUA revelou que:

1) Ainda que os EUA fosse um páis desenvolvido, sofreu grandes impactos na sociedade devido a COVID-19;
2) As populações mais vulneráveis da região de Durham, do estado da Carolina do Norte, tiveram mais casos em relação às populações com determinantes sociais de saúde melhores;

PERSPECTIVAS FUTURAS

Frente aos promissores achados obtidos com as análises espacais tanto na região sul do Brasil, quando na região leste dos EUA, faz-se necessário avaliar como as políticas de saúde nesses locais poderiam contribuir para melhorar o desfecho da população.

Análises adicionais como técnicas de aprendizado de máquinas *(machine learning)* podem auxiliar na clássificação de grupos de grupos mais vulneráveis à COVID-19 e com baixa acessibilidade aos recursos de saúde.

Modelagens utilizando estatística Bayesiana podem fornecer indícios sobre os estimativas riscos de uma população vulnerável evoluir para um desfecho negativo e detectar os locais mais propensos a terem uma falha dos serviços de saúde.

Atualmente técnicas de SIG, como análise espacial e mapeamento, estão desempenhando papéis cada vez mais importante no planejamento para o aumento da acessibilidade à saúde, portanto, é imprescindível que o conhecimento gerado por essas análises cheguem de forma clara e objetiva para os gestores de saúde. Sendo assim, análises mais simples como detecção de clusters e hotsposts poderão ser utilizadas com maior robustes caso as informações pontuais de pacientes possam ser acessadas.

Submission Guidelines

Related information for authors

- PLOS Writing Center
- Submission system
- Journal scope and publication criteria
- Getting started guide
- Guidelines for revisions
- Publication fees
- APC Support

Style and Format

File format	Manuscript files can be in the following formats: DOC, DOCX, or RTF. Microsoft Word documents should not be locked or protected. LaTeX manuscripts must be submitted as PDFs. Read the LaTeX guidelines.
Length	Manuscripts can be any length. There are no restrictions on word count, number of figures, or amount of supporting information. We encourage you to present and discuss your findings concisely.
Font	Use a standard font size and any standard font, except for the font named "Symbol". To add symbols to the manuscript, use the Insert → Symbol function in your word processor or paste in the appropriate Unicode character.
Headings	Limit manuscript sections and sub-sections to 3 heading levels. Make sure heading levels are clearly indicated in the manuscript text.
Layout and spacing	Manuscript text should be double-spaced. Do not format text in multiple columns.
Page and line numbers	Include page numbers and line numbers in the manuscript file. Use continuous line numbers (do not restart the numbering on each page).
Footnotes	Footnotes are not permitted. If your manuscript contains footnotes, move the information into the main text or the reference list, depending on the content.
Language	Manuscripts must be submitted in English. You may submit translations of the manuscript or abstract as supporting information. Read the supporting information guidelines.
Abbreviations	Define abbreviations upon first appearance in the text. Do not use non-standard abbreviations unless they appear at least three times in the text. Keep abbreviations to a minimum.
Reference style	PLOS uses "Vancouver" style, as outlined in the ICMJE sample references. See reference formatting examples and additional instructions below.

Equations	We recommend using MathType for display and inline equations, as it will provide the most reliable outcome. If this is not possible, Equation Editor or Microsoft's Insert→Equation function is acceptable. Avoid using MathType, Equation Editor, or the Insert→Equation function to insert single variables (e.g., "$a^2 + b^2 = c^2$"), Greek or other symbols (e.g., β, Δ, or ′ [prime]), or mathematical operators (e.g., x, ≥, or ±) in running text. Wherever possible, insert single symbols as normal text with the correct Unicode (hex) values. Do not use MathType, Equation Editor, or the Insert→Equation function for only a portion of an equation. Rather, ensure that the entire equation is included. Equations should not contain a mix of different equation tools. Avoid "hybrid" inline or display equations, in which part is text and part is MathType, or part is MathType and part is Equation Editor.
Nomenclature	Use correct and established nomenclature wherever possible.

Units of measurement	Use SI units. If you do not use these exclusively, provide the SI value in parentheses after each value. Read more about SI units.
Drugs	Provide the Recommended International Non-Proprietary Name (rINN).
Species names	Write in italics (e.g., *Homo sapiens*). Write out in full the genus and species, both in the title of the manuscript and at the first mention of an organism in a paper. After first mention, the first letter of the genus name followed by the full species name may be used (e.g., *H. sapiens*).
Genes, mutations, genotypes, and alleles	Write in italics. Use the recommended name by consulting the appropriate genetic nomenclature database (e.g., HGNC for human genes; we strongly recommend using this tool to check against previously approved names). It is sometimes advisable to indicate the synonyms for the gene the first time it appears in the text. Gene prefixes such as those used for oncogenes or cellular localization should be shown in roman typeface (e.g., v-fes, c-MYC).
Allergens	The systematic allergen nomenclature of the World Health Organization/International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature Sub-committee should be used for manuscripts that include the description or use of allergenic proteins. For manuscripts describing new allergens, the systematic name of the allergen should be approved by the WHO/IUIS Allergen Nomenclature Sub-Committee prior to manuscript publication. Examples of the systematic allergen nomenclature can be found at the WHO/IUIS Allergen Nomenclature site.

Copyediting manuscripts

Prior to submission, authors who believe their manuscripts would benefit from professional editing are encouraged to use language-editing and copyediting services. Obtaining this service is the responsibility of the author, and should be done before initial submission. These services can be found on the web using search terms like "scientific editing service" or "manuscript editing service."

Submissions are not copyedited before publication.

Submissions that do not meet the *PLOS ONE* publication criterion for language standards may be rejected.

Manuscript Organization

Manuscripts should be organized as follows. Instructions for each element appear below the list.

Beginning section	*The following elements are required, in order:* › Title page: List title, authors, and affiliations as first page of the manuscript › Abstract › Introduction
Middle section	*The following elements can be renamed as needed and presented in any order:* › Materials and Methods › Results › Discussion › Conclusions (optional)

Ending section	*The following elements are required, in order:* › Acknowledgments › References › Supporting information captions (if applicable)
Other elements	› Figure captions are inserted immediately after the first paragraph in which the figure is cited. Figure files are uploaded separately. › Tables are inserted immediately after the first paragraph in which they are cited. › Supporting information files are uploaded separately.

Refer to our downloadable sample files to ensure that your submission meets our formatting requirements:

› Download sample title, author list, and affiliations page (PDF)
› Download sample manuscript body (PDF)

Viewing Figures and Supporting Information in the compiled submission PDF
The compiled submission PDF includes low-resolution preview images of the figures after the reference list. The function of these previews is to allow you to download the entire submission as quickly as possible. Click the link at the top of each preview page to download a high-resolution version of each figure. Links to download Supporting Information files are also available after the reference list.

Parts of a Submission

Title

Include a full title and a short title for the manuscript.

Title	Length	Guidelines	Examples
Full title	250 characters	Specific, descriptive, concise, and comprehensible to readers outside the field	Impact of cigarette smoke exposure on innate immunity: A *Caenorhabditis elegans* model Solar drinking water disinfection (SODIS) to reduce childhood diarrhoea in rural Bolivia: A cluster-randomized, controlled trial
Short title	100 characters	State the topic of the study	Cigarette smoke exposure and innate immunity SODIS and childhood diarrhoea

Titles should be written in sentence case (only the first word of the text, proper nouns, and genus names are capitalized). Avoid specialist abbreviations if possible. For clinical trials, systematic reviews, or meta-analyses, the subtitle should include the study design.

Author list

Authorship requirements

All authors must meet the criteria for authorship as outlined in the authorship policy. Those who contributed to the work but do not meet the criteria for authorship can be mentioned in the Acknowledgments. Read more about Acknowledgments.

The corresponding author must provide an ORCID iD at the time of submission by entering it in the user profile in the submission system. Read more about ORCID.

Author names and affiliations

Enter author names on the title page of the manuscript and in the online submission system.

On the title page, write author names in the following order:

› First name (or initials, if used)

- Middle name (or initials, if used)
- Last name (surname, family name)

Each author on the list must have an affiliation. The affiliation includes department, university, or organizational affiliation and its location, including city, state/province (if applicable), and country. Authors have the option to include a current address in addition to the address of their affiliation at the time of the study. The current address should be listed in the byline and clearly labeled "current address." At a minimum, the address must include the author's current institution, city, and country.

If an author has multiple affiliations, enter all affiliations on the title page only. In the submission system, enter only the preferred or primary affiliation. Author affiliations will be listed in the typeset PDF article in the same order that authors are listed in the submission.

Author names will be published exactly as they appear in the manuscript file. Please double-check the information carefully to make sure it is correct.

Corresponding author

The submitting author is automatically designated as the corresponding author in the submission system. The corresponding author is the primary contact for the journal office and the only author able to view or change the manuscript while it is under editorial consideration.

The corresponding author role may be transferred to another coauthor. However, note that transferring the corresponding author role also transfers access to the manuscript. (To designate a new corresponding author while the manuscript is still under consideration, watch the video tutorial below.)

Only one corresponding author can be designated in the submission system, but this does not restrict the number of corresponding authors that may be listed on the article in the event of publication. Whoever is designated as a corresponding author on the title page of the manuscript file will be listed as such upon publication. Include an email address for each corresponding author listed on the title page of the manuscript.

How to select a new corresponding author in Editorial Manager

Consortia and group authorship

If a manuscript is submitted on behalf of a consortium or group, include its name in the manuscript byline. Do not add it to the author list in the submission system. You may include the full list of members in the Acknowledgments or in a supporting information file.

PubMed only indexes individual consortium or group author members listed in the article byline. If included, these individuals must qualify for authorship according to our criteria.

Read the group authorship policy.

Author contributions

Provide at minimum one contribution for each author in the submission system. Use the CRediT taxonomy to describe each contribution. Read the policy and the full list of roles.

Contributions will be published with the final article, and they should accurately reflect contributions to the work. The submitting author is responsible for completing this information at submission, and we expect that all authors will have reviewed, discussed, and agreed to their individual contributions ahead of this time.

PLOS ONE will contact all authors by email at submission to ensure that they are aware of the submission.

Cover letter

Upload a cover letter as a separate file in the online system. The length limit is 1 page.

The cover letter should include the following information:

- Summarize the study's contribution to the scientific literature
- Relate the study to previously published work
- Specify the type of article (for example, research article, systematic review, meta-analysis, clinical trial)
- Describe any prior interactions with PLOS regarding the submitted manuscript
- Suggest appropriate Academic Editors to handle your manuscript (see the full list of Academic Editors)
- List any opposed reviewers

IMPORTANT: Do not include requests to reduce or waive publication fees in the cover letter. This information will be entered separately in the online submission system.

Read about publication fee assistance.

Title page

The title, authors, and affiliations should all be included on a title page as the first page of the manuscript file.

Download our sample title, author list, and affiliations page (PDF)

Abstract

The Abstract comes after the title page in the manuscript file. The abstract text is also entered in a separate field in the submission system.

The Abstract should:

- Describe the main objective(s) of the study
- Explain how the study was done, including any model organisms used, without methodological detail
- Summarize the most important results and their significance
- Not exceed 300 words

Abstracts should not include:

- Citations
- Abbreviations, if possible

Introduction

The introduction should:

- Provide background that puts the manuscript into context and allows readers outside the field to understand the purpose and significance of the study
- Define the problem addressed and why it is important
- Include a brief review of the key literature
- Note any relevant controversies or disagreements in the field
- Conclude with a brief statement of the overall aim of the work and a comment about whether that aim was achieved

Materials and Methods

The Materials and Methods section should provide enough detail to allow suitably skilled investigators to fully replicate your study. Specific information and/or protocols for new methods should be included in detail. If materials, methods, and protocols are well established, authors may cite articles where those protocols are described in detail, but the submission should include sufficient information to be understood independent of these references.

Supporting reproducibility with protocols

To enhance the reproducibility of your results, we recommend and encourage you to make your protocols public. There are several options:

Protocols associated with Research Articles

Protocol documents may be uploaded as Supporting Information or linked from the Methods section of the article. For laboratory protocols, we recommend protocols.io. Include the DOI link in the Methods section of your manuscript using the following format: http://dx.doi.org/10.17504/protocols.io.[PROTOCOL DOI]. This allows editors and reviewers to consult the detailed step-by-step protocol when evaluating your manuscript. You can choose to keep the protocol private on the protocols.io platform until your article is published—at which time it will be published automatically.

Protocols published in their own right

PLOS ONE offers two options for publishing stand-alone protocol articles: Lab Protocols that describe reusable methodologies and Study Protocols that describe detailed plans and proposals for research projects. Specific guidelines apply to the submission of Lab Protocol and Study Protocol manuscripts. Read the detailed instructions for submitting Lab Protocols and Study Protocols.

Results, Discussion, Conclusions

These sections may all be separate, or may be combined to create a mixed Results/Discussion section (commonly labeled "Results and Discussion") or a mixed Discussion/Conclusions section (commonly labeled "Discussion"). These sections may be further divided into subsections, each with a concise subheading, as appropriate. These sections have no word limit, but the language should be clear and concise.

Together, these sections should describe the results of the experiments, the interpretation of these results, and the conclusions that can be drawn.

Authors should explain how the results relate to the hypothesis presented as the basis of the study and provide a succinct explanation of the implications of the findings, particularly in relation to previous related studies and potential future directions for research.

PLOS ONE editorial decisions do not rely on perceived significance or impact, so authors should avoid overstating their conclusions. See the *PLOS ONE* Criteria for Publication for more information.

Acknowledgments

Those who contributed to the work but do not meet our authorship criteria should be listed in the Acknowledgments with a description of the contribution.

Authors are responsible for ensuring that anyone named in the Acknowledgments agrees to be named.

PLOS journals publicly acknowledge the indispensable efforts of our editors and reviewers on an annual basis. To ensure equitable recognition and avoid any appearance of partiality, do not include editors or peer reviewers—named or unnamed—in the Acknowledgments.

Do not include funding sources in the Acknowledgments or anywhere else in the manuscript file. Funding information should only be entered in the financial disclosure section of the submission system.

References

Any and all available works can be cited in the reference list. Acceptable sources include:

- Published or accepted manuscripts
- Manuscripts on preprint servers, providing the manuscript has a citable DOI or arXiv URL.

Do not cite the following sources in the reference list:

- Unavailable and unpublished work, including manuscripts that have been submitted but not yet accepted (e.g., "unpublished work," "data not shown"). Instead, include those data as supplementary material or deposit the data in a publicly available database.
- Personal communications (these should be supported by a letter from the relevant authors but not included in the reference list)
- Submitted research should not rely upon retracted research. You should avoid citing retracted articles unless you need to discuss retracted work to provide historical context for your submitted research. If it is necessary to discuss retracted work, state the article's retracted status in your article's text and reference list.

Ensure that your reference list includes full and current bibliography details for every cited work at the time of your article's submission (and publication, if accepted). If cited work is corrected, retracted, or marked with an expression of concern before your article is published, and if you feel it is appropriate to cite the work even in light of the post-publication notice, include in your manuscript citations and full references for both the affected article and the post-publication notice. Email the journal office if you have questions.

References are listed at the end of the manuscript and numbered in the order that they appear in the text. In the text, cite the reference number in square brackets (e.g., "We used the techniques developed by our colleagues [19] to analyze the data"). PLOS uses the numbered citation (citation-sequence) method and first six authors, et al.

Do not include citations in abstracts.

Make sure the parts of the manuscript are in the correct order *before* ordering the citations.

Formatting references

Because all references will be linked electronically as much as possible to the papers they cite, proper formatting of

references is crucial.

PLOS uses the reference style outlined by the International Committee of Medical Journal Editors (ICMJE), also referred to as the "Vancouver" style. Example formats are listed below. Additional examples are in the ICMJE sample references.

A reference management tool, EndNote, offers a current style file that can assist you with the formatting of your references. If you have problems with any reference management program, please contact the source company's technical support.

Journal name abbreviations should be those found in the National Center for Biotechnology Information (NCBI) databases.

Source	Format
Published articles	Hou WR, Hou YL, Wu GF, Song Y, Su XL, Sun B, et al. cDNA, genomic sequence cloning and overexpression of ribosomal protein gene L9 (rpL9) of the giant panda (Ailuropoda melanoleuca). Genet Mol Res. 2011;10: 1576-1588. Devaraju P, Gulati R, Antony PT, Mithun CB, Negi VS. Susceptibility to SLE in South Indian Tamils may be influenced by genetic selection pressure on TLR2 and TLR9 genes. Mol Immunol. 2014 Nov 22. pii: S0161-5890(14)00313-7. doi: 10.1016/j.molimm.2014.11.005. Note: A DOI number for the full-text article is acceptable as an alternative to or in addition to traditional volume and page numbers. When providing a DOI, adhere to the format in the example above with both the label and full DOI included at the end of the reference (doi: 10.1016/j.molimm.2014.11.005). Do not provide a shortened DOI or the URL.
Accepted, unpublished articles	Same as published articles, but substitute "Forthcoming" for page numbers or DOI.
Online articles	Huynen MMTE, Martens P, Hilderlink HBM. The health impacts of globalisation: a conceptual framework. Global Health. 2005;1: 14. Available from: http://www.globalizationandhealth.com/content/1/1/14
Books	Bates B. Bargaining for life: A social history of tuberculosis. 1st ed. Philadelphia: University of Pennsylvania Press; 1992.
Book chapters	Hansen B. New York City epidemics and history for the public. In: Harden VA, Risse GB, editors. AIDS and the historian. Bethesda: National Institutes of Health; 1991. pp. 21-28.
Deposited articles (preprints, e-prints, or arXiv)	Krick T, Shub DA, Verstraete N, Ferreiro DU, Alonso LG, Shub M, et al. Amino acid metabolism conflicts with protein diversity. arXiv:1403.3301v1 [Preprint]. 2014 [cited 2014 March 17]. Available from: https://128.84.21.199/abs/1403.3301v1 Kording KP, Mensh B. Ten simple rules for structuring papers. BioRxiv [Preprint]. 2016 bioRxiv 088278 [posted 2016 Nov 28; revised 2016 Dec 14; revised 2016 Dec 15; cited 2017 Feb 9]: [12 p.]. Available from: https://www.biorxiv.org/content/10.1101/088278v5 doi: 10.1101/088278
Published media (print or online newspapers and magazine articles)	Fountain H. For Already Vulnerable Penguins, Study Finds Climate Change Is Another Danger. The New York Times. 2014 Jan 29 [Cited 2014 March 17]. Available from: http://www.nytimes.com/2014/01/30/science/earth/climate-change-taking-toll-on-penguins-study-finds.html
New media (blogs, web sites, or other written works)	Allen L. Announcing PLOS Blogs. 2010 Sep 1 [cited 17 March 2014]. In: PLOS Blogs [Internet]. San Francisco: PLOS 2006 - . [about 2 screens]. Available from: http://blogs.plos.org/plos/2010/09/announcing-plos-blogs/.
Masters' theses or doctoral dissertations	Wells A. Exploring the development of the independent, electronic, scholarly journal. M.Sc. Thesis, The University of Sheffield. 1999. Available from: http://cumincad.scix.net/cgi-bin/works/Show?2e09
Databases and repositories (Figshare, arXiv)	Roberts SB. QPX Genome Browser Feature Tracks; 2013 [cited 2013 Oct 5]. Database: figshare [Internet]. Available from: http://figshare.com/articles/QPX_Genome_Browser_Feature_Tracks/701214
Multimedia (videos, movies, or TV shows)	Hitchcock A, producer and director. Rear Window [Film]; 1954. Los Angeles: MGM.

Supporting information

Authors can submit essential supporting files and multimedia files along with their manuscripts. All supporting information will be subject to peer review. All file types can be submitted, but files must be smaller than 20 MB in size.

Authors may use almost any description as the item name for a supporting information file as long as it contains an "S" and number. For example, "S1 Appendix" and "S2 Appendix," "S1 Table" and "S2 Table," and so forth.

Supporting information files are published exactly as provided, and are not copyedited.

Supporting information captions

List supporting information captions at the end of the manuscript file. Do not submit captions in a separate file.

The file number and name are required in a caption, and we highly recommend including a one-line title as well. You may also include a legend in your caption, but it is not required.

Example caption

S1 Text. Title is strongly recommended. Legend is optional.

In-text citations

We recommend that you cite supporting information in the manuscript text, but this is not a requirement. If you cite supporting information in the text, citations do not need to be in numerical order.

Read the supporting information guidelines for more details about submitting supporting information and multimedia files.

Figures and tables

Figures

Do not include figures in the main manuscript file. Each figure must be prepared and submitted as an individual file.

Cite figures in ascending numeric order at first appearance in the manuscript file.

Read the guidelines for figures and requirements for reporting blot and gel results.

Figure captions

Figure captions must be inserted in the text of the manuscript, immediately following the paragraph in which the figure is first cited (read order). Do not include captions as part of the figure files themselves or submit them in a separate document.

At a minimum, include the following in your figure captions:

- A figure label with Arabic numerals, and "Figure" abbreviated to "Fig" (e.g. Fig 1, Fig 2, Fig 3, etc). Match the label of your figure with the name of the file uploaded at submission (e.g. a figure citation of "Fig 1" must refer to a figure file named "Fig1.tif").
- A concise, descriptive title

The caption may also include a legend as needed.

Read more about figure captions.

Tables

Cite tables in ascending numeric order upon first appearance in the manuscript file.

Place each table in your manuscript file directly after the paragraph in which it is first cited (read order). Do not submit your tables in separate files.

Tables require a label (e.g., "Table 1") and brief descriptive title to be placed above the table. Place legends, footnotes, and other text below the table.

Read the guidelines for tables.

Statistical reporting

Manuscripts submitted to *PLOS ONE* are expected to report statistical methods in sufficient detail for others to replicate the analysis performed. Ensure that results are rigorously reported in accordance with community standards and that statistical methods employed are appropriate for the study design.

Consult the following resources for additional guidance:

- SAMPL guidelines, for general guidance on statistical reporting
- *PLOS ONE* guidelines, for clinical trials requirements
- *PLOS ONE* guidelines, for systematic review and meta-analysis requirements
- EQUATOR, for specific reporting guidelines for a range of other study types

Reporting of statistical methods

In the methods, include a section on statistical analysis that reports a detailed description of the statistical methods. In this section:

- List the name and version of any software package used, alongside any relevant references
- Describe technical details or procedures required to reproduce the analysis
- Provide the repository identifier for any code used in the analysis (See our code-sharing policy.)

Statistical reporting guidelines:

- Identify research design and independent variables as being between- or within-subjects
- For pre-processed data:
  - Describe any analysis carried out to confirm the data meets the assumptions of the analysis performed (e.g. linearity, co-linearity, normality of the distribution).
  - If data were transformed include this information, with a reason for doing so and a description of the transformation performed
- Provide details of how outliers were treated and your analysis, both with the full dataset and with the outliers removed
- If relevant, describe how missing/excluded data were handled
- Define the threshold for significance (alpha)
- If appropriate, provide sample sizes, along with a description of how they were determined. If a sample size calculation was performed, specify the inputs for power, effect size and alpha. Where relevant, report the number of independent replications for each experiment.
- For analyses of variance (ANOVAs), detail any post hoc tests that were performed
- Include details of any corrections applied to account for multiple comparisons. If corrections were not applied, include a justification for not doing so
- Describe all options for statistical procedures. For example, if t-tests were performed, state whether these were one- or two-tailed. Include details of the type of t-test conducted (e.g. one sample, within-/between-subjects).
- For step-wise multiple regression analyses:
  - Report the alpha level used
  - Discuss whether the variables were assessed for collinearity and interaction
  - Describe the variable selection process by which the final model was developed (e.g., forward-stepwise; best subset). See SAMPL guidelines.
- For Bayesian analysis explain the choice of prior trial probabilities and how they were selected. Markov chain Monte Carlo settings should be reported.

Reporting of statistical results

Results must be rigorously and appropriately reported, in keeping with community standards.

- **Units of measurement.** Clearly define measurement units in all tables and figures.
- **Properties of distribution.** It should be clear from the text which measures of variance (standard deviation, standard error of the mean, confidence intervals) and central tendency (mean, median) are being presented.
- **Regression analyses.** Include the full results of any regression analysis performed as a supplementary file. Include all estimated regression coefficients, their standard error, p-values, and confidence intervals, as well as the measures of goodness of fit.
- **Reporting parameters.** Test statistics (F/t/r) and associated degrees of freedom should be provided. Effect sizes and confidence intervals should be reported where appropriate. If percentages are provided, the numerator and denominator should also be given.
- **P-values.** Report exact p-values for all values greater than or equal to 0.001. P-values less than 0.001 may be expressed as $p < 0.001$, or as exponentials in studies of genetic associations.
- **Displaying data in plots.** Format plots so that they accurately depict the sample distribution. 3D effects in plots can bias and hinder interpretation of values, so avoid them in cases where regular plots are sufficient to display the data.
- **Open data.** As explained in PLOS's Data Policy, be sure to make individual data points, underlying graphs and summary statistics available at the time of publication. Data can be deposited in a repository or included within the Supporting Information files.

Data reporting

All data and related metadata underlying the findings reported in a submitted manuscript should be deposited in an appropriate public repository, unless already provided as part of the submitted article.

See instructions on providing underlying data to support blot and gel results.

Read our policy on data availability.

Repositories may be either subject-specific (where these exist) and accept specific types of structured data, or generalist repositories that accept multiple data types. We recommend that authors select repositories appropriate to their field. Repositories may be subject-specific (e.g., GenBank for sequences and PDB for structures), general, or institutional, as long as DOIs or accession numbers are provided and the data are at least as open as CC BY. Authors are encouraged to select repositories that meet accepted criteria as trustworthy digital repositories, such as criteria of the Centre for Research Libraries or Data Seal of Approval. Large, international databases are more likely to persist than small, local ones.

See our list of recommended repositories.

To support data sharing and author compliance of the PLOS data policy, we have integrated our submission process with a select set of data repositories. The list is neither representative nor exhaustive of the suitable repositories available to authors. Current repository integration partners include Dryad and FlowRepository. Please contact data@plos.org to make recommendations for further partnerships.

Instructions for PLOS submissions with data deposited in an integration partner repository:

- Deposit data in the integrated repository of choice.
- Once deposition is final and complete, the repository will provide you with a dataset DOI (provisional) and private URL for reviewers to gain access to the data.
- Enter the given data DOI into the full Data Availability Statement, which is requested in the Additional Information section of the PLOS submission form. Then provide the URL passcode in the Attach Files section.

If you have any questions, please email us.

Accession numbers

All appropriate data sets, images, and information should be deposited in an appropriate public repository. See our list of recommended repositories.

Accession numbers (and version numbers, if appropriate) should be provided in the Data Availability Statement. Accession numbers or a citation to the DOI should also be provided when the data set is mentioned within the manuscript.

In some cases authors may not be able to obtain accession numbers of DOIs until the manuscript is accepted; in these cases, the authors must provide these numbers at acceptance. In all other cases, these numbers must be provided at full submission.

Identifiers

As much as possible, please provide accession numbers or identifiers for all entities such as genes, proteins, mutants, diseases, etc., for which there is an entry in a public database, for example:

- Ensembl
- Entrez Gene
- FlyBase
- InterPro
- Mouse Genome Database (MGD)
- Online Mendelian Inheritance in Man (OMIM)
- PubChem

Identifiers should be provided in parentheses after the entity on first use.

Striking image

You can choose to upload a “Striking Image” that we may use to represent your article online in places like the journal homepage or in search results.

The striking image must be derived from a figure or supporting information file from the submission, i.e., a cropped portion of an image or the entire image. Striking images should ideally be high resolution, eye-catching, single panel images, and should ideally avoid containing added details such as text, scale bars, and arrows.

If no striking image is uploaded, we will designate a figure from the submission as the striking image.

Striking images should not contain potentially identifying images of people. Read our policy on identifying information.

The PLOS licenses and copyright policy also applies to striking images.

Additional Information Requested at Submission

Financial Disclosure Statement

This information should describe sources of funding that have supported the work. It is important to gather these details prior to submission because your financial disclosure statement cannot be changed after initial submission without journal approval. If your manuscript is published, your statement will appear in the Funding section of the article.

Enter this statement in the Financial Disclosure section of the submission form. Do not include it in your manuscript file.

The statement should include:

- Specific grant numbers
- Initials of authors who received each award
- Full names of commercial companies that funded the study or authors
- Initials of authors who received salary or other funding from commercial companies
- URLs to sponsors' websites

Also state whether any sponsors or funders (other than the named authors) played any role in:

- Study design
- Data collection and analysis
- Decision to publish
- Preparation of the manuscript

If they had no role in the research, include this sentence: "The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript."

If the study was unfunded, include this sentence as the Financial Disclosure statement: "The author(s) received no specific funding for this work."

Read our policy on disclosure of funding sources.

Competing interests

This information should not be in your manuscript file; you will provide it via our submission system.

All potential competing interests must be declared in full. If the submission is related to any patents, patent applications, or products in development or for market, these details, including patent numbers and titles, must be disclosed in full.

Read our policy on competing interests.

Manuscripts disputing published work

For manuscripts disputing previously published work, it is *PLOS ONE* policy to invite a signed review by the disputed author during the peer review process. This procedure is aimed at ensuring a thorough, transparent, and productive review process.

If the disputed author chooses to submit a review, it must be returned in a timely fashion and contain a full declaration of all competing interests. The Academic Editor will consider any such reviews in light of the competing interest.

Authors submitting manuscripts disputing previous work should explain the relationship between the manuscripts in their cover letter, and will be required to confirm that they accept the conditions of this review policy before the manuscript is considered further.

Related manuscripts

Upon submission, authors must confirm that the manuscript, or any related manuscript, is not currently under consideration or accepted elsewhere. If related work has been submitted to *PLOS ONE* or elsewhere, authors must include a copy with the submitted article. Reviewers will be asked to comment on the overlap between related submissions.

We strongly discourage the unnecessary division of related work into separate manuscripts, and we will not consider manuscripts that are divided into "parts." Each submission to *PLOS ONE* must be written as an independent unit and should not rely on any work that has not already been accepted for publication. If related manuscripts are submitted to *PLOS ONE*, the authors may be advised to combine them into a single manuscript at the editor's discretion.

Read our policies on related manuscripts.

Preprints

PLOS encourages authors to post preprints to accelerate the dissemination of research. Posting a manuscript on a preprint server does not impact consideration of the manuscript at any PLOS journal.

Authors posting preprints on bioRxiv or medRxiv can choose to concurrently submit their manuscripts to relevant PLOS journals through the direct transfer service.

Authors submitting manuscripts in the life and health sciences to *PLOS ONE* may choose to have PLOS forward their submission to bioRxiv or medRxiv, depending on the scope of the paper, for consideration for posting as a preprint.

Read more about preprints.

Learn how to post a preprint to bioRxiv or medRxiv at *PLOS ONE*.

Guidelines for Specific Study Types

Study design, reporting, and analyses are assessed against all relevant research and methodological technique standards held by the community. Guidelines for specific study types are outlined below.

Registered Reports

Submission and format requirements for Registered Report Protocols and Registered Reports are similar to those for a regular submission and may be specific to your study type. For instance, if your Registered Report Protocol submission is about a Clinical Trial or a Systematic Review, follow the appropriate guidelines.

For Registered Report Protocols:

- Provide enough methodological detail to make the study reproducible and replicable
- Confirm that data will be made available upon study completion in keeping with the PLOS Data policy
- Include ethical approval or waivers, if applicable
- Preliminary or pilot data may be included, but only if necessary to support the feasibility of the study or as a proof of principle
- For meta-analyses or Clinical Trials, use the protocol-specific reporting guidelines PRISMA-P or SPIRIT respectively

For more guidance on format and presentation of a protocol, consult the sample template hosted by the Open Science Framework. Discipline-specific and study-specific templates are also available.

If data need to be collected, modified or processed specifically for your study, or if participants need to be recruited specifically for your study, then it should occur only after your Registered Report Protocol is accepted for publication.

For Registered Report Research Articles:

- Report the results of all planned analyses and, if relevant, detail and justify all deviations from the protocol.
- The manuscript may also contain exploratory, unplanned analyses.

Read more about Registered Report framework.

Human subjects research

All research involving human participants must have been approved by the authors' Institutional Review Board (IRB) or by equivalent ethics committee(s), and must have been conducted according to the principles expressed in the Declaration of Helsinki. Authors should be able to submit, upon request, a statement from the IRB or ethics committee indicating approval of the research. We reserve the right to reject work that we believe has not been conducted to a high ethical standard, even when formal approval has been obtained.

Subjects must have been properly instructed and have indicated that they consent to participate by signing the appropriate informed consent paperwork. Authors may be asked to submit a blank, sample copy of a subject consent form. If consent was verbal instead of written, or if consent could not be obtained, the authors must explain the reason in the manuscript, and the use of verbal consent or the lack of consent must have been approved by the IRB or ethics committee.

All efforts should be made to protect patient privacy and anonymity. Identifying information, including photos, should not be included in the manuscript unless the information is crucial and the individual has provided written consent by completing the Consent Form for Publication in a PLOS Journal (PDF). Download additional translations of the form here. More information about patient privacy, anonymity, and informed consent can be found in the International Committee of Medical Journal Editors (ICMJE) Privacy and Confidentiality guidelines.

Manuscripts should conform to the following reporting guidelines:

- Studies of diagnostic accuracy: STARD
- Observational studies: STROBE

- Microarray experiments: MIAME
- Other types of health-related research: Consult the EQUATOR web site for appropriate reporting guidelines

Methods sections of papers on research using human subjects or samples must include ethics statements that specify:

- **The name of the approving institutional review board or equivalent committee(s)**. If approval was not obtained, the authors must provide a detailed statement explaining why it was not needed
- **Whether informed consent was written or oral**. If informed consent was oral, it must be stated in the manuscript:
  - Why written consent could not be obtained
  - That the Institutional Review Board (IRB) approved use of oral consent
  - How oral consent was documented

For studies involving humans categorized by race/ethnicity, age, disease/disabilities, religion, sex/gender, sexual orientation, or other socially constructed groupings, authors should:

- Explicitly describe their methods of categorizing human populations
- Define categories in as much detail as the study protocol allows
- Justify their choices of definitions and categories, including for example whether any rules of human categorization were required by their funding agency
- Explain whether (and if so, how) they controlled for confounding variables such as socioeconomic status, nutrition, environmental exposures, or similar factors in their analysis

In addition, outmoded terms and potentially stigmatizing labels should be changed to more current, acceptable terminology. Examples: "Caucasian" should be changed to "white" or "of [Western] European descent" (as appropriate); "cancer victims" should be changed to "patients with cancer."

For papers that include identifying, or potentially identifying, information, authors must download the Consent Form for Publication in a PLOS Journal, which the individual, parent, or guardian must sign once they have read the paper and been informed about the terms of PLOS open-access license. The signed consent form should not be submitted with the manuscript, but authors should securely file it in the individual's case notes and the methods section of the manuscript should explicitly state that consent authorization for publication is on file, using wording like:

The individual in this manuscript has given written informed consent (as outlined in PLOS consent form) to publish these case details.

For more information about *PLOS ONE* policies regarding human subjects research, see the Publication Criteria and Editorial Policies.

Manuscripts describing observational clinical studies are subject to all policies regarding human research and community standards for reporting observational research as outlined by the STROBE statement. Furthermore, authors submitting work of this nature should pay special attention to the following requirements:

- If the submitted manuscript is very similar to previous work, authors must provide a sound scientific rationale for the submitted work and clearly reference and discuss the existing literature.
- The sampling strategy and eligibility criteria of enrolled subjects should be described in sufficient detail.
- Sample size calculations should be justified with relevant inputs defined.
- Independent and dependent variables considered for statistical analysis should be clearly defined and justified.
- The validity and reliability testing of self-developed data collection tools should be reported.
- Conclusions should be appropriate for the study design, with indications on how the study results will contribute to the base of academic knowledge.

Clinical trials

Clinical trials are subject to all policies regarding human research. *PLOS ONE* follows the World Health Organization's (WHO) definition of a clinical trial:

> *A clinical trial is any research study that prospectively assigns human participants or groups of humans to one or more health-related interventions to evaluate the effects on health outcomes [...] Interventions include but are not restricted to drugs, cells and other biological products, surgical procedures, radiologic procedures, devices, behavioural treatments, process-of-care changes, preventive care, etc.*

All clinical trials must be registered in one of the publicly-accessible registries approved by the WHO or ICMJE (International Committee of Medical Journal Editors). Authors must provide the trial registration number. Prior disclosure of results on a clinical trial registry site will not affect consideration for publication. We reserve the right to inform authors' institutions or ethics committees, and to reject the manuscript, if we become aware of unregistered trials.

PLOS ONE supports prospective trial registration (i.e. before participant recruitment has begun) as recommended by the ICMJE's clinical trial registration policy. **Where trials were not publicly registered before participant recruitment began**, authors must:

- Register all related clinical trials and confirm they have done so in the Methods section
- Explain in the Methods the reason for failing to register before participant recruitment

Clinical trials must be reported according to the relevant reporting guidelines, i.e. CONSORT for randomized controlled trials, TREND for non-randomized trials, and other specialized guidelines as appropriate. The intervention should be described according to the requirements of the TIDieR checklist and guide. Submissions must also include the study protocol as supporting information, which will be published with the manuscript if accepted.

Authors of manuscripts describing the results of clinical trials must adhere to the CONSORT reporting guidelines appropriate to their trial design, available on the CONSORT Statement web site. Before the paper can enter peer review, authors must:

- The name of the registry and the registration number must be included in the Abstract.
- Provide a copy of the trial protocol as approved by the ethics committee and a completed CONSORT checklist as supporting information (which will be published alongside the paper, if accepted). This should be named S1 CONSORT Checklist.
- Include the CONSORT flow diagram as the manuscript's "Fig 1"

Any deviation from the trial protocol must be explained in the paper. Authors must explicitly discuss informed consent in their paper, and we reserve the right to ask for a copy of the patient consent form.

The name of the registry and the registry number must be provided in the Abstract. If the trial is registered in more than one location, please provide all relevant registry names and numbers.

Lab Protocols

Lab Protocols consist of two interlinked components: a step-by-step protocol hosted on protocols.io, and a peer-reviewed article in *PLOS ONE* that contextualises the protocol.

protocols.io is a secure platform for developing and sharing reproducible methods. It enables scientists to make, exchange, improve, and discuss protocols for specific experimental procedures. The platform provides specialized tools for communicating technical details, including reagents, measurements, and formulae.

The *PLOS ONE* article component must comply with the general *PLOS ONE* submission guidelines (detailed above) and criteria for publication. In addition, the *PLOS ONE* article component should:

- Describe the value that the protocol adds to the published literature. **Lab Protocols describing routine methods or extensions and modifications of routine methods that add little value to the published literature will not be considered for publication.**
- Provide evidence that the protocol works, by either:
  - Linking, in the Introduction section, to at least one supporting peer-reviewed publication in which the protocol was applied to generate data.
    or
  - Providing validation or benchmarking data, which demonstrates that the underlying method achieves its intended purpose.
- Provide the step-by-step protocol as a supporting information (S1) file.

Download a Lab Protocol article template

We encourage you to post your protocol to the protocols.io platform before submitting your manuscript to *PLOS ONE*. **Posting your protocol prior to submission is not considered prior publication by *PLOS ONE* and will not affect your eligibility to publish a Lab Protocol.**

Authors submitting a Lab Protocol can also use protocols.io's protocol entry service at no cost: the team at protocols.io will enter your protocol for you and format it in a way that takes advantage of the platform's features. You will have an opportunity to review and make further changes before your protocol is shared with anyone else.

If you would like to use protocols.io's protocol entry service in connection with a Lab Protocol submission, please contact plosone@plos.org to request the customer code.

If you prefer to submit your manuscript to *PLOS ONE* before posting your protocol to protocols.io, then you must still provide your step-by-step protocol as a supporting information (S1) file in a format of your choosing. You will be expected to replace this file with a protocols.io PDF later in the editorial process.

Study Protocols

Study Protocols describe plans for conducting research projects and consist of a single article on *PLOS ONE*.

Study Protocols must comply with the *PLOS ONE* general submission guidelines (detailed above in this article) and any guidelines specific to the related research study type. In addition, the protocol must:

- Relate to a research study that has not yet generated results.
- Be submitted before recruitment of participants or collection of data for the study is complete.
- Meet the same standards for ethics of experimentation and research integrity as the research study. If it involves human or

animal subjects, cell lines or field sampling, or has potential biosafety implications, prior approval from the relevant ethics body must be obtained prior to submission. Please contact us if you have a valid reason for not obtaining approval.

Additional prerequisites apply for these study types:

- Clinical trials:
  - The trial must be registered prior to submission of your protocol in one of the publicly accessible registries approved by the WHO or ICMJE (International Committee of Medical Journal Editors).
  - The name of the registry and the trial or study registration number must be included in the Abstract.
  - A copy of the protocol that was approved by the ethics committee must be submitted as a supplementary information file. Please provide an additional English translation if the original document is not in English. Please note that the protocol will be published with the manuscript if accepted.
  - A SPIRIT schedule of enrollment, interventions, and assessments must be included as the manuscript's Figure 1, and a completed SPIRIT checklist must be uploaded as Supporting Information file S1.
- Systematic reviews and meta-analyses:
  - A completed PRISMA-P checklist must be provided as a supporting information (SI) file. See PRISMA-P Explanation and Elaboration for more information on completing your checklist.

Study Protocols must also comply with general *PLOS ONE* criteria for publication and in addition you should:

- include the word "Protocol" in your Title.
- include a detailed description of the planned study in the Materials and Methods section.This should provide sufficient methodological detail for the protocol to be reproducible and replicable. Your description should cover all relevant and applicable facts and hypothesis, including:
  - the aim, design, and settling
  - the sample size calculation
  - how data saturation will be determined (for qualitative studies)
  - the characteristics of participants e.g., inclusion and exclusion criteria, sample selection criteria, variables to be measured, randomization and blinding criteria (where applicable), and how informed consent will be obtained
  - how materials will be selected and used e.g., where and how they will be sourced, the processes, interventions, or comparisons to be used, the outcomes to be measured, and when and how they will be measured
  - the data management plan
  - safety considerations
  - the type of data and statistical analyses to be used
  - the status and timeline of the study, including whether participant recruitment or data collection has begun
  - where and when the data will be made available. See our Data Availability policy for more.
- include an analysis of preliminary or pilot data, only if it is necessary to support the feasibility of the study or as a proof of principle. This is optional.
- we encourage authors you to register with OSF and provide the your registration number in the Materials and Methods section. This is optional.
- optionally add any other SI files, figures or tables that elaborate or authenticate the protocol: e.g., any reporting checklists applicable to your study type.

Read the supporting information guidelines for more details about adding SI files.

Download our sample Study Protocol template or an OSF discipline or study-specific template.

Study Protocols are subject to the same editorial and peer review process as all other articles, and are eligible for both signed and published peer review.

You can expedite the review process by providing:

- proof of external funding. This is typically your funding approval letter and a list of the names and credentials of the funders who conducted the external peer review of the protocol. Include an English translation if needed.
- proof of ethics approval (if required). This is typically the approval or waiver letter from the relevant ethics body and a copy of the protocol approved by this body.

The proof of external funding and approval or waiver letter are used for internal purposes and do not form part of the published Study Protocol.
Expedited review is conducted by an internal Staff Editor only and bypasses the external review process.

If the Study Protocol describes a replication study or involves re-analysis of published work, we will invite the author of the initial or replicated study to provide a signed review.

We encourage you to share your Study Protocol with other researchers, either before or after submission. You can publish it on your website or protocols.io, or submit it for posting on medRxiv or another preprint server.

Animal research

All research involving vertebrates or cephalopods must have approval from the authors' Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee(s), and must have been conducted according to applicable national and international guidelines. Approval must be received prior to beginning research.

Manuscripts reporting animal research must state in the Methods section:

- The full name of the relevant ethics committee that approved the work, and the associated permit number(s).
- Where ethical approval is not required, the manuscript should include a clear statement of this and the reason why. Provide any relevant regulations under which the study is exempt from the requirement for approval.
- Relevant details of steps taken to ameliorate animal suffering.

Example ethics statement

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of the University of Minnesota (Protocol Number: 27-2956). All surgery was performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering.

Authors should always state the organism(s) studied in the Abstract. Where the study may be confused as pertaining to clinical research, authors should also state the animal model in the title.

To maximize reproducibility and potential for re-use of data, we encourage authors to follow the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines for all submissions describing laboratory-based animal research and to upload a completed ARRIVE Guidelines Checklist to be published as supporting information.

Non-human primates

Manuscripts describing research involving non-human primates must report details of husbandry and animal welfare in accordance with the recommendations of the Weatherall report, *The use of non-human primates in research*, including:

- Information about housing, feeding, and environmental enrichment.
- Steps taken to minimize suffering, including use of anesthesia and method of sacrifice, if appropriate.

Random source animals

Manuscripts describing studies that use random source (e.g. Class B dealer-sourced in the USA), shelter, or stray animals will be subject to additional scrutiny and may be rejected if sufficient ethical and scientific justification for the study design is lacking.

Unacceptable euthanasia methods and anesthetic agents

Manuscripts reporting use of a euthanasia method(s) classified as unacceptable by the American Veterinary Medical Association or use of an anesthesia method(s) that is widely prohibited (e.g., chloral hydrate, ether, chloroform) must include at the time of initial submission, scientific justification for use in the specific study design, as well as confirmation of approval for specific use from their animal research ethics committee. These manuscripts may be subject to additional ethics considerations prior to publication.

Humane endpoints

Manuscripts reporting studies in which death of a regulated animal (vertebrate, cephalopod) is a likely outcome or a planned experimental endpoint, must comprehensively report details of study design, rationale for the approach, and methodology, including consideration of humane endpoints. This applies to research that involves, for instance, assessment of survival, toxicity, longevity, terminal disease, or high rates of incidental mortality.

Definition of a humane endpoint

A humane endpoint is a predefined experimental endpoint at which animals are euthanized when they display early markers associated with death or poor prognosis of quality of life, or specific signs of severe suffering or distress. Humane endpoints are used as an alternative to allowing such conditions to continue or progress to death following the experimental intervention ("death as an endpoint"), or only euthanizing animals at the end of an experiment. Before a study begins, researchers define the practical observations or measurements that will be used during the study to recognize a humane endpoint, based on anticipated clinical, physiological, and behavioral signs. Please see the NC3Rs guidelines for more information. Additional discussion of humane endpoints can be found in this article: Nuno H. Franco, Margarida Correia-

Neves, I. Anna S. Olsson (2012) How "Humane" Is Your Endpoint? — Refining the Science-Driven Approach for Termination of Animal Studies of Chronic Infection. PLoS Pathog 8(1): e1002399 doi.org/10.1371/journal.ppat.1002399.

Full details of humane endpoints use must be reported for a study to be reproducible and for the results to be accurately interpreted.

For studies in which death of an animal is an outcome or a planned experimental endpoint, authors should include the following information in the Methods section of the manuscript:

- The specific criteria (i.e. humane endpoints) used to determine when animals should be euthanized.
- The duration of the experiment.
- The numbers of animals used, euthanized, and found dead (if any); the cause of death for all animals.
- How frequently animal health and behavior were monitored.
- All animal welfare considerations taken, including efforts to minimize suffering and distress, use of analgesics or anaesthetics, or special housing conditions.

If humane endpoints were not used, the manuscript should report:

- A scientific justification for the study design, including the reasons why humane endpoints could not be used, and discussion of alternatives that were considered.
- Whether the institutional animal ethics committee specifically reviewed and approved the anticipated mortality in the study design.

Observational and field studies

Methods sections for submissions reporting on any type of field study must include ethics statements that specify:

- Permits and approvals obtained for the work, including the full name of the authority that approved the study; if none were required, authors should explain why
- Whether the land accessed is privately owned or protected
- Whether any protected species were sampled
- Full details of animal husbandry, experimentation, and care/welfare, where relevant

Paleontology and archaeology research

Manuscripts reporting paleontology and archaeology research must include descriptions of methods and specimens in sufficient detail to allow the work to be reproduced. Data sets supporting statistical and phylogenetic analyses should be provided, preferably in a format that allows easy re-use. Read the policy.

Specimen numbers and complete repository information, including museum name and geographic location, are required for publication. Locality information should be provided in the manuscript as legally allowable, or a statement should be included giving details of the availability of such information to qualified researchers.

If permits were required for any aspect of the work, details should be given of all permits that were obtained, including the full name of the issuing authority. This should be accompanied by the following statement:

All necessary permits were obtained for the described study, which complied with all relevant regulations.

If no permits were required, please include the following statement:

No permits were required for the described study, which complied with all relevant regulations.

Manuscripts describing paleontology and archaeology research are subject to the following policies:

- **Sharing of data and materials.** Any specimen that is erected as a new species, described, or figured must be deposited in an accessible, permanent repository (i.e., public museum or similar institution). If study conclusions depend on specimens that do not fit these criteria, the article will be rejected under *PLOS ONE*'s data availability criterion.
- **Ethics.** *PLOS ONE* will not publish research on specimens that were obtained without necessary permission or were illegally exported.

Systematic reviews and meta-analyses

A systematic review paper, as defined by The Cochrane Collaboration, is a review of a clearly formulated question that uses explicit, systematic methods to identify, select, and critically appraise relevant research, and to collect and analyze data from the studies that are included in the review. These reviews differ substantially from narrative-based reviews or synthesis articles. Statistical methods (meta-analysis) may or may not be used to analyze and summarize the results of the included studies.

Reports of systematic reviews and meta-analyses should include a completed PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) checklist and flow diagram to accompany the

main text. Blank templates are available here.

- Checklist: PDF or Word document
- Flow diagram: PDF or Word document

Authors must also state in their "Methods" section whether a protocol exists for their systematic review, and if so, provide a copy of the protocol as supporting information and provide the registry number in the abstract.

If your article is a systematic review or a meta-analysis you should:

- State this in your cover letter
- Select "Research Article" as your article type when submitting
- Include the PRISMA flow diagram as Fig 1 (required where applicable)
- Include the PRISMA checklist as supporting information

Meta-analysis of genetic association studies

Manuscripts reporting a meta-analysis of genetic association studies must report results of value to the field and should be reported according to the guidelines presented in *Systematic Reviews of Genetic Association Studies* by Sagoo *et al*.

On submission, authors will be asked to justify the rationale for the meta-analysis and how it contributes to the base of scientific knowledge in the light of previously published results. Authors will also be asked to complete a checklist (DOCX) outlining information about the justification for the study and the methodology employed. Meta-analyses that replicate published studies will be rejected if the authors do not provide adequate justification.

Personal data from third-party sources

For all studies using personal data from internet-based and other third-party sources (e.g., social media, blogs, other internet sources, mobile phone companies), data must be collected and used according to company/website Terms and Conditions, with appropriate permissions. All data sources must be acknowledged clearly in the Materials and Methods section.

Read our policy on data availability.

In the Ethics Statement, authors should declare any potential risks to individuals or individual privacy, or affirm that in their assessment, the study posed no such risks. In addition, the following Ethics and Data Protection requirements must be met.

For interventional studies, which impact participants' experiences or data, the study design must have been prospectively approved by an Ethics Committee, and informed consent is required. The Ethics Committee may waive the requirement for approval and/or consent.

For observational studies in which personal experiences and accounts are not manipulated, consultation with an Ethics or Data Protection Committee is recommended. Additional requirements apply in the following circumstances:

- If information used could threaten personal privacy or damage the reputation of individuals whose data are used, an Ethics Committee should be consulted and informed consent obtained or specifically addressed.
- If authors accessed any personal identifying information, an Ethics or Data Protection Committee should oversee data anonymization. If data were anonymized and/or aggregated before access and analysis, informed consent is generally not required.

Note that Terms of Use contracts do not qualify as informed consent, even if they address the use of personal data for research.

See our reporting guidelines for human subjects research.

Cell lines

Authors reporting research using cell lines should state when and where they obtained the cells, giving the date and the name of the researcher, cell line repository, or commercial source (company) who provided the cells, as appropriate.

Authors must also include the following information for each cell line:

For *de novo* (new) cell lines, including those given to the researchers as a gift, authors must follow our policies for human subjects research or animal research, as appropriate. The ethics statement must include:

- Details of institutional review board or ethics committee approval; AND
- For human cells, confirmation of written informed consent from the donor, guardian, or next of kin

For established cell lines, the Methods section should include:

- A reference to the published article that first described the cell line; AND/OR
- The cell line repository or company the cell line was obtained from, the catalogue number, and whether the cell line was obtained directly from the repository/company or from another laboratory

Authors should check established cell lines using the ICLAC Database of Cross-contaminated or Misidentified Cell Lines to confirm they are not misidentified or contaminated. Cell line authentication is recommended – e.g., by karyotyping, isozyme analysis, or short tandem repeats (STR) analysis – and may be required during peer review or after publication.

Blots and gels

Please review *PLOS ONE's* requirements for reporting blot and gel results and providing the underlying raw images.

Antibodies

Manuscripts reporting experiments using antibodies should include the following information:

- The name of each antibody, a description of whether it is monoclonal or polyclonal, and the host species.
- The commercial supplier or source laboratory.
- The catalogue or clone number and, if known, the batch number.
- The antigen(s) used to raise the antibody.
- For established antibodies, a stable public identifier from the Antibody Registry.

The manuscript should also report the following experimental details:

- The final antibody concentration or dilution.
- A reference to the validation study if the antibody was previously validated. If not, provide details of how the authors validated the antibody for the applications and species used.

We encourage authors to consider adding information on new validations to a publicly available database such as Antibodypedia or CiteAb.

Small and macromolecule crystal data

Manuscripts reporting new and unpublished three-dimensional structures must include sufficient supporting data and detailed descriptions of the methodologies used to allow the reproduction and validation of the structures. All novel structures must have been deposited in a community endorsed database prior to submission (please see our list of recommended repositories).

Small molecule single crystal data

Authors reporting X-Ray crystallographic structures of small organic, metal-organic, and inorganic molecules must deposit their data with the Cambridge Crystallographic Data Centre (CCDC), the Inorganic Crystal Structure Database (ICSD), or similar community databases providing a recognized validation functionality. Authors are also required to include the relevant structure reference numbers within the main text (e.g. the CCDC ID number), as well as the crystallographic information files (.cif format) as Supplementary Information, along with the checkCIF validation reports that can be obtained via the International Union of Crystallography (IUCr).

Macromolecular structures

Authors reporting novel macromolecular structures must have deposited their data prior to initial submission with the Worldwide Protein Data Bank (wwPDB), the Biological Magnetic Resonance Data Bank (BMRB), the Electron Microscopy Data Bank (EMDB), or other community databases providing a recognized validation functionality. Authors must include the structure reference numbers within the main text and submit as Supplementary Information the official validation reports from these databases.

Methods, software, databases, and tools

PLOS ONE will consider submissions that present new methods, software, databases, or tools as the primary focus of the manuscript if they meet the following criteria:

Utility
The tool must be of use to the community and must present a proven advantage over existing alternatives, where applicable. Recapitulation of existing methods, software, or databases is not useful and will not be considered for publication. Combining data and/or functionalities from other sources may be acceptable, but simpler instances (i.e. presenting a subset of an already existing database) may not be considered. For software, databases, and online tools, the long-term utility should also be discussed, as relevant. This discussion may include maintenance, the potential for future growth, and the stability of the hosting, as applicable.

Validation
Submissions presenting methods, software, databases, or tools must demonstrate that the new tool achieves its intended purpose. If similar options already exist, the submitted manuscript must demonstrate that the new tool is an improvement over existing options in some way. This requirement may be met by including a proof-of-principle experiment or analysis; if

this is not possible, a discussion of the possible applications and some preliminary analysis may be sufficient.

Availability
If the manuscript's primary purpose is the description of new software or a new software package, this software must be open source, deposited in an appropriate archive, and conform to the Open Source Definition. If the manuscript mainly describes a database, this database must be open-access and hosted somewhere publicly accessible, and any software used to generate a database should also be open source. If relevant, databases should be open for appropriate deposition of additional data. Dependency on commercial software such as Mathematica and MATLAB does not preclude a paper from consideration, although complete open source solutions are preferred. In these cases, authors should provide a direct link to the deposited software or the database hosting site from within the paper. If the primary focus of a manuscript is the presentation of a new tool, such as a newly developed or modified questionnaire or scale, it should be openly available under a license no more restrictive than CC BY.

Software submissions

Manuscripts whose primary purpose is the description of new software must provide full details of the algorithms designed. Describe any dependencies on commercial products or operating system. Include details of the supplied test data and explain how to install and run the software. A brief description of enhancements made in the major releases of the software may also be given. Authors should provide a direct link to the deposited software from within the paper.

Database submissions

For descriptions of databases, provide details about how the data were curated, as well as plans for long-term database maintenance, growth, and stability. Authors should provide a direct link to the database hosting site from within the paper.

Read the PLOS policy on sharing materials, software and code.

New taxon names

Zoological names

When publishing papers that describe a new zoological taxon name, PLOS aims to comply with the requirements of the International Commission on Zoological Nomenclature (ICZN). Effective 1 January 2012, the ICZN considers an online-only publication to be legitimate if it meets the criteria of archiving and is registered in ZooBank, the ICZN's official registry.

For proper registration of a new zoological taxon, we require two specific statements to be included in your manuscript.

In the **Results** section, the globally unique identifier (GUID), currently in the form of a Life Science Identifier (LSID), should be listed under the new species name, for example:

Anochetus boltoni *Fisher* ***sp. nov.*** *urn:lsid:zoobank.org:act:B6C072CF-1CA6-40C7-8396-534E91EF7FBB*

You will need to contact Zoobank to obtain a GUID (LSID). Please do this as early as possible to avoid delay of publication upon acceptance of your manuscript. It is your responsibility to provide us with this information so we can include it in the final published paper.

Please also insert the following text into the **Methods** section, in a sub-section to be called "Nomenclatural Acts":

The electronic edition of this article conforms to the requirements of the amended International Code of Zoological Nomenclature, and hence the new names contained herein are available under that Code from the electronic edition of this article. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix "http://zoobank.org/". The LSID for this publication is: urn:lsid:zoobank.org:pub: XXXXXXX. The electronic edition of this work was published in a journal with an ISSN, and has been archived and is available from the following digital repositories: LOCKSS [author to insert any additional repositories].

All PLOS articles are deposited in LOCKSS. If your institute, or those of your co-authors, has its own repository, we recommend that you also deposit the published online article there and include the name in your article.

Botanical names

When publishing papers that describe a new botanical taxon, PLOS aims to comply with the requirements of the International Code of Nomenclature for algae, fungi, and plants (ICN). The following guidelines for publication in an online-only journal have been agreed such that any scientific botanical name published by us is considered effectively published under the rules of the Code. Please note that these guidelines differ from those for zoological nomenclature, and apply only to seed plants, ferns, and lycophytes.

Effective January 2012, the description or diagnosis of a new taxon can be in either Latin or English. This does not affect the requirements for scientific names, which are still to be Latin.

Also effective January 2012, the electronic PDF represents a published work according to the ICN for algae, fungi, and plants. Therefore the new names contained in the electronic publication of PLOS article are effectively published under that Code from the electronic edition alone, so there is no longer any need to provide printed copies.

Additional information describing recent changes to the Code can be found here.

For proper registration of the new taxon, we require two specific statements to be included in your manuscript.

In the **Results** section, the globally unique identifier (GUID), currently in the form of a Life Science Identifier (LSID), should be listed under the new species name, for example:

> **Solanum aspersum** S.Knapp, sp. nov. [urn:lsid:ipni.org:names:77103633-1] Type: Colombia. Putumayo: vertiente oriental de la Cordillera, entre Sachamates y San Francisco de Sibundoy, 1600-1750 m, 30 Dec 1940, J. Cuatrecasas 11471 (holotype, COL; isotypes, F [F-1335119], US [US-1799731]).

Journal staff will contact IPNI to obtain the GUID (LSID) after your manuscript is accepted for publication, and this information will then be added to the manuscript during the production phase

In the **Methods** section, include a sub-section called "Nomenclature" using the following wording:

> The electronic version of this article in Portable Document Format (PDF) in a work with an ISSN or ISBN will represent a published work according to the International Code of Nomenclature for algae, fungi, and plants, and hence the new names contained in the electronic publication of a PLOS article are effectively published under that Code from the electronic edition alone, so there is no longer any need to provide printed copies.
>
> In addition, new names contained in this work have been submitted to IPNI, from where they will be made available to the Global Names Index. The IPNI LSIDs can be resolved and the associated information viewed through any standard web browser by appending the LSID contained in this publication to the prefix http://ipni.org/. The online version of this work is archived and available from the following digital repositories: [INSERT NAMES OF DIGITAL REPOSITORIES WHERE ACCEPTED MANUSCRIPT WILL BE SUBMITTED (LOCKSS etc)].

All PLOS articles are deposited in LOCKSS. If your institute, or those of your co-authors, has its own repository, we recommend that you also deposit the published online article there and include the name in your article.

Fungal names

When publishing papers that describe a new botanical taxon, PLOS aims to comply with the requirements of the International Code of Nomenclature for algae, fungi, and plants (ICN). The following guidelines for publication in an online-only journal have been agreed such that any scientific botanical name published by us is considered effectively published under the rules of the Code. Please note that these guidelines differ from those for zoological nomenclature.

Effective January 2012, the description or diagnosis of a new taxon can be in either Latin or English. This does not affect the requirements for scientific names, which are still to be Latin.

Also effective January 2012, the electronic PDF represents a published work according to the ICN for algae, fungi, and plants. Therefore the new names contained in the electronic publication of PLOS article are effectively published under that Code from the electronic edition alone, so there is no longer any need to provide printed copies.

Additional information describing recent changes to the Code can be found here.

For proper registration of the new taxon, we require two specific statements to be included in your manuscript.

In the **Results** section, the globally unique identifier (GUID), currently in the form of a Life Science Identifier (LSID), should be listed under the new species name, for example:

> ***Hymenogaster huthii.*** Stielow et al. 2010, sp. nov. [urn:lsid:indexfungorum.org:names:518624]

You will need to contact either Mycobank or Index Fungorum to obtain the GUID (LSID). Please do this as early as possible to avoid delay of publication upon acceptance of your manuscript. It is your responsibility to provide us with this information so we can include it in the final published paper. Effective January 2013, all papers describing new fungal species must reference the identifier issued by a recognized repository in the protologue in order to be considered effectively published.

In the **Methods** section, include a sub-section called "Nomenclature" using the following wording. Note that this example is for taxon names submitted to MycoBank; please substitute appropriately if you have submitted to Index Fungorum using the prefix http://www.indexfungorum.org/Names /NamesRecord.asp?RecordID=.

> The electronic version of this article in Portable Document Format (PDF) in a work with an ISSN or ISBN will represent a published work according to the International Code of Nomenclature for algae, fungi, and plants, and hence the new names contained in the electronic publication of a PLOS article are effectively published under that Code from the electronic edition alone, so there is no longer any need to provide printed copies.

In addition, new names contained in this work have been submitted to MycoBank from where they will be made available to the Global Names Index. The unique MycoBank number can be resolved and the associated information viewed through any standard web browser by appending the MycoBank number contained in this publication to the prefix http://www.mycobank.org/MB/. The online version of this work is archived and available from the following digital repositories: [INSERT NAMES OF DIGITAL REPOSITORIES WHERE ACCEPTED MANUSCRIPT WILL BE SUBMITTED (LOCKSS etc)].

All PLOS articles are deposited in LOCKSS. If your institute, or those of your co-authors, has its own repository, we recommend that you also deposit the published online article there and include the name in your article.

Qualitative research

Qualitative research studies use non-quantitative methods to address a defined research question that may not be accessible by quantitative methods, such as people's interpretations, experiences, and perspectives. The analysis methods are explicit, systematic, and reproducible, but the results do not involve numerical values or use statistics. Examples of qualitative data sources include, but are not limited to, interviews, text documents, audio/video recordings, and free-form answers to questionnaires and surveys.

Qualitative research studies should be reported in accordance to the Consolidated criteria for reporting qualitative research (COREQ) checklist or Standards for reporting qualitative research (SRQR) checklist. Further reporting guidelines can be found in the Equator Network's Guidelines for reporting qualitative research.

You may be eligible for APC support

Many institutional partners globally have publishing agreements with PLOS to allow their corresponding authors to publish with reduced or no APCs. To determine if your corresponding author is eligible, please visit our institutional partners page to determine what kind of agreement your institution has with PLOS.

If your corresponding author is affiliated with a participating institution, they must follow the instructions below to demonstrate eligibility.

Read the full instructions for submitting to a journal with the Flat Fee Agreement.

If your corresponding author is not from a participating institution and requires assistance paying publishing fees, please consider applying for a fee waiver at submission.