AMERICAN SOCIETY FOR MICROBIOLOGY | mBio

| Microbial Ecology | Commentary

Leveraging the human microbiota to target bacterial respiratory pathogens: new paths toward an expanded antimicrobial armamentarium

Jillian H. Hurst,[1,2] **Matthew S. Kelly**[1,2]

AUTHOR AFFILIATIONS See affiliation list on p. 4.

ABSTRACT Acute respiratory infections are the most frequent infections across the lifespan and are the leading infectious cause of death among children globally. Bacterial respiratory infections are routinely treated with antibiotics, nearly all of which are derived from microbial natural products. Unfortunately, antibiotic-resistant bacteria are an increasingly frequent cause of respiratory infections, and there are few new antibiotics in development that target these pathogens. In the article by Stubbendieck et al., the authors identified *Rothia* species that demonstrate *in vitro* and *ex vivo* growth inhibition of the respiratory pathobiont *Moraxella catarrhalis*. The authors present experiments suggesting that this activity is mediated at least in part through the secretion of a novel peptidoglycan endopeptidase that targets the *M. catarrhalis* cell wall. In this commentary, we discuss these findings in the context of the urgent threat of antimicrobial resistance and highlight the promise of the human respiratory microbiota as a source of novel biotherapeutics.

KEYWORDS *Moraxella catarrhalis*, antibiotic resistance, human microbiome, respiratory pathogens, comparative genomics, *Rothia* species, microbial ecology, acute otitis media

Acute respiratory infections are the most common infections at all ages and are associated with substantial morbidity and mortality. Globally, these infections account for nearly four million deaths each year, most of which result from pneumonia and occur in low- and middle-income countries (LMICs) (1). Other respiratory infections, including acute otitis media (AOM) and acute bacterial rhinosinusitis, are rarely life-threatening but have an enormous societal burden. For example, more than five million cases of AOM occur each year among children in the United States resulting in direct health expenditures exceeding $6 billion (2). The development and deployment of vaccines against *Streptococcus pneumoniae* and *Haemophilus influenzae* type b have been a cornerstone of recent public health efforts to reduce the global burden of respiratory infections. However, these vaccines are only effective in preventing infection caused by vaccine serotypes, and vaccine introduction has been followed by the emergence of non-vaccine serotype strains that are highly virulent and multidrug resistant. Additionally, while these vaccines have been associated with a reduction in respiratory infections such as AOM, the proportion of these infections caused by *M. catarrhalis* and other pathobionts for which there is no licensed vaccine has increased (3, 4).

M. catarrhalis is a Gram-negative, nonencapsulated diplococcus that colonizes the upper respiratory tracts of 30%–100% of infants and 1%–5% of adults. Although the majority of individuals colonized by *M. catarrhalis* are asymptomatic, this species is a leading cause of AOM, chronic obstructive pulmonary disease (COPD) exacerbations, and bacterial rhinosinusitis (5). There are currently no licensed vaccines for *M. catarrhalis*;

Editor Joerg Graf, University of Connecticut, Storrs, Connecticut, USA

Address correspondence to Matthew S. Kelly, matthew.kelly@duke.edu.

The authors declare no conflict of interest.

See the funding table on p. 4.

Published 20 June 2023

moreover, a leading vaccine candidate was recently found to be ineffective in preventing moderate or severe COPD exacerbations, although efficacy studies have not been conducted in other conditions or among children (6). Antibiotic resistance is also a significant concern in treating infections caused by *M. catarrhalis*. Approximately 90% of strains are resistant to amoxicillin, the first-line antibiotic for respiratory infections among children, with increasing resistance to macrolides and fluoroquinolones (7). Considering the substantial role of this species in respiratory infections and the negative consequences of the use of broad-spectrum antibiotics, effective vaccines and new therapeutics are urgently needed for *M. catarrhalis*.

In the article by Stubbendieck and colleagues (8), the authors found that the abundance of the bacterial genus *Rothia* was negatively correlated with *M. catarrhalis* carriage in a small cohort of children. After cultivating *Rothia* strains from nasal samples from these children, the authors screened these strains for inhibition of *M. catarrhalis* growth. They then used a combination of comparative genomics and proteomics to identify potential mechanisms underlying this activity, leading ultimately to the identification and purification of a putative endopeptidase, SagA, which was demonstrated to degrade *M. catarrhalis* peptidoglycan. Finally, the authors showed that strains of two *Rothia* species, *Rothia aeria* and a potential novel species provisionally named *R. similmucilaginosa*, reduce colonization of human respiratory epithelial cells by *M. catarrhalis*. Here, we seek to provide context to these findings and their importance to efforts to combat antimicrobial resistance and develop novel therapeutics to prevent or treat respiratory infections.

The overuse and misuse of antibiotics in humans and livestock and increased human mobility have fueled the rapid expansion and global dissemination of antibiotic resistance determinants. In the United States alone, antimicrobial-resistant organisms cause more than 2.8 million infections and 35,000 deaths each year (9). Over the past decade, notable progress has been made in slowing the spread of some antimicrobial-resistant organisms; however, antibiotic resistance to several common bacterial respiratory pathogens rose substantially during this time period. In particular, increasing resistance of *S. pneumoniae* to beta-lactam antibiotics and macrolides was observed globally, with the prevalence of multidrug-resistant (non-susceptible to ≥3 antibiotic classes) and extensively drug-resistant (non-susceptible to ≥5 antibiotic classes) strains in some regions approaching 50% and 20%, respectively (10). Similarly, several countries reported a high and rising prevalence of both multidrug-resistant and extensively drug-resistant *H. influenzae* strains (11, 12). Although antibiotic resistance affects all countries, the burden is higher in the world's poorest nations. Nearly 90% of deaths attributable to bacterial antibiotic resistance, including more than 99.6% of child deaths, occur in LMICs (13). A 2016 analysis estimated that antibiotic resistance will result in more than 10 million deaths annually by 2050, underscoring the critical need for development of new antibiotics (14).

Clinically relevant bacteria have developed substantial resistance to most existing classes of antibiotics, making identification of new antibiotic classes vital to addressing the growing threat of antimicrobial resistance. Between 1930 and 1962—often referred to as "the golden age of antibiotics"—more than 20 classes of antibiotics were brought to market in the United States (15). Unfortunately, these early successes in antibiotic development led to the widespread belief that antibiotics would eliminate infectious diseases which, when combined with the high costs of research and development, discouraged pharmaceutical companies from further investments in the antibiotic pipeline. As a result, only two new antibiotic classes were approved over the subsequent 50 y. Recent efforts, including the U.S. National Action Plan for Combating Antibiotic-Resistant Bacteria, have contributed substantial resources to accelerate antibiotic development. Unfortunately, as highlighted by a 2021 report by the World Health Organization, gaps in the preclinical and clinical antibiotic pipelines remain, with most drugs in development being analogues of existing antibiotics (16).

The vast majority of antibiotics currently in use are microbial natural products or their derivatives. Historically, antibiotic development efforts focused on soil-based actinomycetes and involved screening of strains for zones of growth inhibition on petri dishes overlaid with various pathogens. As a result, actinomycetes are the source of many of the antibiotics used in modern medicine, including tetracyclines, aminoglycosides, macrolides, rifamycins, and vancomycin. Unfortunately, this untargeted approach to antibiotic development was labor intensive and yielded few candidate compounds relative to the number of strains screened, with identification of new antibiotic classes from actinomycetes becoming increasingly difficult over time. In the 1990s, antibiotic development efforts shifted largely to screening libraries of synthetic compounds for activity against defined bacterial targets. However, this approach proved largely unsuccessful, in part because of the challenge of identifying synthetic compounds that penetrate bacterial cells, and was ultimately abandoned by most pharmaceutical companies. The diminishing returns or failures of these previous antibiotic development platforms highlight the need for exploration of new sources of antibiotics and use of targeted approaches to identifying candidate compounds.

The human microbiota represents a vast and largely untapped source of novel antibiotics. The microbes that reside in and on our bodies have coevolved with humans and with each other, resulting in both symbiotic and antagonistic relationships among species occupying the same ecological niche. Among the cellular and molecular mechanisms underpinning these complex and bidirectional interactions is the production of antimicrobial compounds that specifically target competing species, including common human pathogens. The development of next-generation sequencing methods and improved tools for genomic and proteomic analysis enables a targeted and biologically informed approach to mining these interspecies relationships for novel antibiotics.

Stubbendieck and colleagues applied just such an approach to identify bacterial strains and a secreted protein with activity against *M. catarrhalis*. Using microbiome data generated from healthy children and children with upper respiratory symptoms, the authors identified a negative association between the relative abundance of *Rothia* and *M. catarrhalis* colonization. After demonstrating that specific *Rothia* strains inhibited *M. catarrhalis* growth at a distance and reasoning that the inhibitory factor was likely to be a protein, the authors further narrowed their search using comparative analyses of the genomes and secreted proteomes of these *Rothia* strains. This led to the identification of a putative peptidoglycan endopeptidase as the lead candidate for the observed activity against *M. catarrhalis*, with subsequent heterologous expression of a truncated form of this protein confirming its inhibition of *M. catarrhalis* growth. Critically, these *Rothia* strains inhibited three *Moraxella* species but not a panel of other Gammaproteobacteria, suggesting that the off-target effects of antimicrobial compounds secreted by these strains may be limited. However, further studies are needed to specifically determine the spectrum of activity of the endopeptidase SagA.

With the growing threat of antimicrobial resistance, other microbiome-based therapies are increasingly being explored as alternatives to antibiotics for infection prevention and treatment. A growing body of evidence indicates that commensal microbes in the upper respiratory tract influence the risk of colonization and infection by bacterial respiratory pathobionts (17, 18). The article by Stubbendieck and colleagues adds to this literature in supporting a role for *Rothia* species in colonization resistance to *M. catarrhalis*, although the presented microbiome analyses are limited by the small sample size and should be evaluated in other studies. Building upon prior observational studies, several recent clinical trials evaluated live bacteria for the elimination or prevention of respiratory pathobiont carriage. Deasy et al. reported that a single intranasal administration of a strain of *Neisseria lactamica* resulted in the eradication of and long-term protection from *N. meningitidis* colonization among university students (19). Similarly, Uehara et al. demonstrated that *S. aureus* colonization could be eradicated in most nasal carriers through intranasal administration of a *Corynebacterium* strain

(20). These studies suggest that further elucidation of interspecies interactions within the human upper respiratory tract could lead to the development of nasal probiotics representing a new paradigm for respiratory infection prevention.

In conclusion, the human microbiota represents a largely unexplored source of novel biotherapeutics for infection prevention and treatment. The study by Stubbendieck and colleagues provides an example of how recent advances, including next-generation sequencing and improved methods for genomic and proteomic analyses, can accelerate development of rationally designed antibiotics. Such highly targeted approaches focused on the human microbiota could identify biotherapeutics that expand our toolset for combatting antibiotic resistance and for precisely engineering the microbiome for the promotion of human health.

ACKNOWLEDGMENTS

M.S.K. was supported by a National Institutes of Health Career Development Award (K23-AI135090). The views and opinions expressed by the authors do not necessarily represent those of the NIH.

AUTHOR AFFILIATIONS

[1]Department of Pediatrics, Division of Infectious Diseases, Duke University School of Medicine, Durham, North Carolina, USA
[2]Duke Microbiome Center, Duke University School of Medicine, Durham, North Carolina, USA

AUTHOR ORCIDs

Matthew S. Kelly http://orcid.org/0000-0001-8819-2315

FUNDING

Funder	Grant(s)	Author(s)
HHS | National Institutes of Health (NIH)	K23-AI135090	Matthew Kelly

AUTHOR CONTRIBUTIONS

Jillian H. Hurst, Writing – original draft, Writing – review and editing.

REFERENCES

1. Vos T, Lim SS, Abbafati C, Abbas KM, Abbasi M, Abbasifard M, Abbasi-Kangevari M, Abbastabar H, Abd-Allah F, Abdelalim A, Abdollahi M, Abdollahpour I, Abolhassani H, Aboyans V, Abrams EM, Abreu LG, Abrigo MRM, Abu-Raddad LJ, Abushouk AI, Acebedo A, Ackerman IN, Adabi M, Adamu AA, Adebayo OM, Adekanmbi V, Adelson JD, Adetokunboh OO, Adham D, Afshari M, Afshin A, Agardh EE, Agarwal G, Agesa KM, Aghaali M, Aghamir SMK, Agrawal A, Ahmad T, Ahmadi A, Ahmadi M, Ahmadieh H, Ahmadpour E, Akalu TY, Akinyemi RO, Akinyemiju T, Akombi B, Al-Aly Z, Alam K, Alam N, Alam S, Alam T, Alanzi TM, Albertson SB, Alcalde-Rabanal JE, Alema NM, Ali M, Ali S, Alicandro G, Alijanzadeh M, Alinia C, Alipour V, Aljunid SM, Alla F, Allebeck P, Almasi-Hashiani A, Alonso J, Al-Raddadi RM, Altirkawi KA, Alvis-Guzman N, Alvis- Z, Amini S, Amini-Rarani M, Aminorroaya A, Amiri F, Amit AML, Amugsi DA, Amul GGH, Anderlini D, Andrei CL, Andrei T, Anjomshoa M, Ansari F, Ansari I, Ansari-M, Antonio CAT, Antony CM, Antriyandarti E, Anvari D, Anwer R, Arabloo J, Arab-Zozani M, Aravkin AY, Ariani F, Ärnlöv J, Aryal KK, Arzani A, Asadi-Aliabadi M, Asadi-Pooya AA, Asghari B, Ashbaugh C, Atnafu DD, Atre SR, Ausloos F, Ausloos M, Quintanilla BPA, Ayano G, Ayanore MA, Aynalem YA, Azari S, Azarian G, Azene ZN, Babaee E, Badawi A, Bagherzadeh M, Bakhshaei MH, Bakhtiari A, Balakrishnan S, Balalla S, Balassyano S, Banach M, Banik PC, Bannick MS, Bante AB, Baraki AG, Barboza MA, Barker-Collo SL, Barthelemy CM, Barua L, Barzegar A, Basu S, Baune BT, Bayati M, Bazmandegan G, Bedi N, Beghi E, Béjot Y, Bello AK, Bender RG, Bennett DA, Bennitt FB, Bensenor IM, Benziger CP, Berhe K, Bernabe E, Bertolacci GJ, Bhageerathy R, Bhala N, Bhandari D, Bhardwaj P, Bhattacharyya K, Bhutta ZA, Bibi S, Biehl MH, Bikbov B, Sayeed MSB, Biondi A, Birihane BM, Bisanzio D, Bisignano C, Biswas RK, Bohlouli S, Bohluli M, Bolla SRR, Boloor A, Boon-Dooley AS, Borges G, Borzì AM, Bourne R, Brady OJ, Brauer M, Brayne C, Breitborde NJK, Brenner H, Briant PS, Briggs AM, Briko NI, Britton GB, Bryazka D, Buchbinder R, Bumgarner BR, Busse R, Butt ZA, Santos FLC, Cámera LLA, Campos-Nonato IR, Car J, Cárdenas R, Carreras G, Carrero JJ, Carvalho F, Castaldelli-Maia JM, Castañeda-Orjuela CA, Castelpietra G, Castle CD, Castro F, Catalá- L, Causey K, Cederroth CR, Cercy KM, Cerin E, Chandan JS, Chang AR, Charlson FJ, Chattu VK, Chaturvedi S, Chimed-Ochir O, Chin KL, Cho DY, Christensen H, Chu DT, Chung MT, Cicuttini FM, Ciobanu LG, Cirillo M, Collins EL, Compton K, Conti S, Cortesi PA, Costa VM, Cousin E, Cowden RG, Cowie BC, Cromwell EA, Cross DH, Crowe CS, Cruz JA, Cunningham M, Dahlawi SMA, Damiani G, Dandona L, Dandona R, Darwesh AM, Daryani A, Das JK, Gupta RD, Neves J, Dávila-Cervantes CA, Davletov K, Leo DD, Dean FE, DeCleene NK, Deen A, Degenhardt L, Dellavalle RP, Demeke FM, Demsie DG, Denova-Gutiérrez E, Dereje ND, Dervenis N, Desai R, Desalew A, Dessie GA, Dharmaratne SD, Dhungana GP, Dianatinasab M, Diaz D, Forooshani ZSD, Dingels ZV, Dirac MA, Djalalinia S, Do HT, Dokova K, Dorostkar F, Doshi CP, Doshmangir L, Douiri A, Doxey MC, Driscoll TR, Dunachie SJ, Duncan BB, Duraes AR, Eagan AW, Kalan ME, Edvardsson D, Ehrlich JR, Nahas NE, Sayed IE, Tantawi ME, Elbarazi I, Elgendy IY, Elhabashy HR, El-Jaafary SI, Elyazar IR,

Emamian MH, Emmons-Bell S, Erskine HE, Eshrati B, Eskandarieh S, Esmaeilnejad S, Esmaeilzadeh F, Esteghamati A, Estep K, Etemadi A, Etisso AE, Farahmand M, Faraj A, Fareed M, Faridnia R, Farinha CS, Farioli A, Faro A, Faruque M, Farzadfar F, Fattahi N, Fazlzadeh M, Feigin VL, Feldman R, Fereshtehnejad S-M, Fernandes E, Ferrari AJ, Ferreira ML, Filip I, Fischer F, Fisher JL, Fitzgerald R, Flohr C, Flor LS, Foigt NA, Folayan MO, Force LM, Fornari C, Foroutan M, Fox JT, Freitas M, Fu W, Fukumoto T, Furtado JM, Gad MM, Gakidou E, Galles NC, Gallus S, Gamkrelidze A, Garcia-Basteiro AL, Gardner WM, Geberemariyam BS, Gebrehiwot AM, Gebremedhin KB, Gebreslassie A, Hayoon AG, Gething PW, Ghadimi M, Ghadiri K, Ghafourifard M, Ghajar A, Ghamari F, Ghashghaee A, Ghiasvand H, Ghith N, Gholamian A, Gilani SA, Gill PS, Gitimoghaddam M, Giussani G, Goli S, Gomez RS, Gopalani SV, Gorini G, Gorman TM, Gottlich HC, Goudarzi H, Goulart AC, Goulart BNG, Grada A, Grivna M, Grosso G, Gubari MIM, Gugnani HC, Guimaraes ALS, Guimarães RA, Guled RA, Guo G, Guo Y, Gupta R, Haagsma JA, Haddock B, Hafezi- N, Hafiz A, Hagins H, Haile LM, Hall BJ, Halvaei I, Hamadeh RR, Abdullah KH, Hamilton EB, Han C, Han H, Hankey GJ, Haro JM, Harvey JD, Hasaballah AI, Hasanzadeh A, Hashemian M, Hassanipour S, Hassankhani H, Havmoeller RJ, Hay RJ, Hay SI, Hayat K, Heidari B, Heidari G, Heidari-Soureshjani R, Hendrie D, Henrikson HJ, Henry NJ, Herteliu C, Heydarpour F, Hird TR, Hoek HW, Hole MK, Holla R, Hoogar P, Hosgood HD, Hosseinzadeh M, Hostiuc M, Hostiuc S, Househ M, Hoy DG, Hsairi M, Hsieh VC, Hu G, Huda TM, Hugo FN, Huynh CK, Hwang B-F, Iannucci VC, Ibitoye SE, Ikuta KS, Ilesanmi OS, Ilic IM, Ilic MD, Inbaraj LR, Ippolito H, Irvani SSN, Islam MM, Islam M, Islam SMS, Islami F, Iso H, Ivers RQ, Iwu CCD, Iyamu IO, Jaafari J, Jacobsen KH, Jadidi-Niaragh F, Jafari H, Jafarinia M, Jahagirdar D, Jahani MA, Jahanmehr N, Jakovljevic M, Jalali A, Jalilian F, James SL, Janjani H, Janodia MD, Jayatilleke AU, Jeemon P, Jenabi E, Jha RP, Jha V, Ji JS, Jia P, John O, John-Akinola YO, Johnson CO, Johnson SC, Jonas JB, Joo T, Joshi A, Jozwiak JJ, Jürisson M, Kabir A, Kabir Z, Kalani H, Kalani R, Kalankesh LR, Kalhor R, Kamiab Z, Kanchan T, Matin BK, Karch A, Karim MA, Karimi SE, Kassa GM, Kassebaum NJ, Katikireddi SV, Kawakami N, Kayode GA, Keddie SH, Keller C, Kereselidze M, Khafaie MA, Khalid N, Khan M, Khatab K, Khater MM, Khatib MN, Khayamzadeh M, Khodayari MT, Khundkar R, Kianipour N, Kieling C, Kim D, Kim Y-E, Kim YJ, Kimokoti RW, Kisa A, Kisa S, Kissimova-Skarbek K, Kivimäki M, Kneib CJ, Knudsen AKS, Kocarnik JM, Kolola T, Kopec JA, Kosen S, Koul PA, Koyanagi A, Kravchenko MA, Krishan K, Krohn KJ, Defo BK, Bicer BK, Kumar GA, Kumar M, Kumar P, Kumar V, Kumaresh G, Kurmi OP, Kusuma D, Kyu HH, Vecchia CL, Lacey B, Lal DK, Lalloo R, Lam JO, Lami FH, Landires I, Lang JJ, Lansingh VC, Larson SL, Larsson AO, Lasrado S, Lassi ZS, Lau K-M, Lavados PM, Lazarus JV, Ledesma JR, Lee PH, Lee SWH, LeGrand KE, Leigh J, Leonardi M, Lescinsky H, Leung J, Levi M, Lewington S, Li S, Lim L-L, Lin C, Lin R-T, Linehan C, Linn S, Liu H-C, Liu S, Liu Z, Looker KJ, Lopez AD, Lopukhov PD, Lorkowski S, Lotufo PA, Lucas TCD, Lugo A, Lunevicius R, Lyons RA, Ma J, MacLachlan JH, Maddison ER, Maddison R, Madotto F, Mahasha PW, Mai HT, Majeed A, Maled V, Maleki S, Malekzadeh R, Malta DC, Mamun AA, Manafi A, Manafi N, Manguerra H, Mansouri B, Mansournia MA, Herrera AMM, Maravilla JC, Marks A, Martins-Melo FR, Martopullo I, Masoumi SZ, Massano J, Massenburg BB, Mathur MR, Maulik PK, McAlinden C, McGrath JJ, McKee M, Mehndiratta MM, Mehri F, Mehta KM, Meitei WB, Memiah PTN, Mendoza W, Menezes RG, Mengesha EW, Mengesha MB, Mereke A, Meretoja A, Meretoja TJ, Mestrovic T, Miazgowski B, Miazgowski T, Michalek IM, Mihretie KM, Miller TR, Mills EJ, Mirica A, Mirrakhimov EM, Mirzaei H, Mirzaei M, Mirzaei-Alavijeh M, Misganaw AT, Mithra P, Moazen B, Moghadaszadeh M, Mohamadi E, Mohammad DK, Mohammad Y, Mezerji NMG, Mohammadian-Hafshejani A, Mohammadifard N, Mohammadpourhodki R, Mohammed S, Mokdad AH, Molokhia M, Momen NC, Monasta L, Mondello S, Mooney MD, Moosazadeh M, Moradi G, Moradi M, Moradi-Lakeh M, Moradzadeh R, Moraga P, Morales L, Morawska L, Velásquez IM, Morgado-da-Costa J, Morrison SD, Mosser JF, Mouodi S, Mousavi SM, Khaneghah AM, Mueller UO, Munro SB, Muriithi MK, Musa KI, Muthupandian S, Naderi M, Nagarajan AJ, Nagel G, Naghshtabrizi B, Nair S, Nandi AK, Nangia V, Nansseu JR, Nayak VC, Nazari J, Negoi I, Negoi RI, Netsere HBN, Ngunjiri JW, Nguyen CT, Nguyen J, Nguyen M, Nguyen M, Nichols E, Nigatu D, Nigatu YT, Nikbakhsh R, Nixon MR, Nnaji CA, Nomura S, Norrving B, Noubiap JJ, Nowak C, Nunez- S, Oţoiu A, Oancea B, Odell CM, Ogbo FA, Oh I-H, Okunga EW, Oladnabi M, Olagunju AT, Olusanya BO, Olusanya JO, Oluwasanu MM, Bali AO, Omer MO, Ong KL, Onwujekwe OE, Orji AU, Orpana HM, Ortiz A, Ostroff SM, Otstavnov N, Otstavnov SS, Øverland S, Owolabi MO, A MP, Padubidri JR, Pakhare AP, Palladino R, Pana A, Panda- J, Pandey A, Park E-K, Parmar PGK, Pasupula DK, Patel SK, Paternina-Caicedo AJ, Pathak A, Pathak M, Patten SB, Patton GC, Paudel D, Toroudi HP, Peden AE, Pennini A, Pepito VCF, Peprah EK, Pereira A, Pereira DM, Perico N, Pham HQ, Phillips MR, Pigott DM, Pilgrim T, Pilz TM, Pirsaheb M, Plana-Ripoll O, Plass D, Pokhrel KN, Polibin RV, Polinder S, Polkinghorne KR, Postma MJ, Pourjafar H, Pourmalek F, Kalhori RP, Pourshams A, Poznańska A, Prada SI, Prakash V, Pribadi DRA, Pupillo E, Syed ZQ, Rabiee M, Rabiee N, Radfar A, Rafiee A, Rafiei A, Raggi A, Rahimi-Movaghar A, Rahman MA, Rajabpour-Sanati A, Rajati F, Ramezanzadeh K, Ranabhat CL, Rao PC, Rao SJ, Rasella D, Rastogi P, Rathi P, Rawaf DL, Rawaf S, Rawal L, Razo C, Redford SB, Reiner RC, Reinig N, Reitsma MB, Remuzzi G, Renjith V, Renzaho AMN, Resnikoff S, Rezaei N, Rezai M, Rezapour A, Rhinehart P-A, Riahi SM, Ribeiro ALP, Ribeiro DC, Ribeiro D, Rickard J, Roberts NLS, Roberts S, Robinson SR, Roever L, Rolfe S, Ronfani L, Roshandel G, Roth GA, Rubagotti E, Rumisha SF, Sabour S, Sachdev PS, Saddik B, Sadeghi E, Sadeghi M, Saeidi S, Safi S, Safiri S, Sagar R, Sahebkar A, Sahraian MA, Sajadi SM, Salahshoor MR, Salamati P, Zahabi SS, Salem H, Salem MRR, Salimzadeh H, Salomon JA, Salz I, Samad Z, Samy AM, Sanabria J, Santomauro DF, Santos IS, Santos JV, Santric-Milicevic MM, Saraswathy SYI, Sarmiento-Suárez R, Sarrafzadegan N, Sartorius B, Sarveazad A, Sathian B, Sathish T, Sattin D, Sbarra AN, Schaeffer LE, Schiavolin S, Schmidt MI, Schutte AE, Schwebel DC, Schwendicke F, Senbeta AM, Senthilkumaran S, Sepanlou SG, Shackelford KA, Shadid J, Shahabi S, Shaheen AA, Shaikh MA, Shalash AS, Shams-Beyranvand M, Shamsizadeh M, Shannawaz M, Sharafi K, Sharara F, Sheena BS, Sheikhtaheri A, Shetty RS, Shibuya K, Shiferaw WS, Shigematsu M, Shin JI, Shiri R, Shirkoohi R, Shrime MG, Shuval K, Siabani S, Sigfusdottir ID, Sigurvinsdottir R, Silva JP, Simpson KE, Singh A, Singh JA, Skiadaresi E, Skou ST, Skryabin VY, Sobngwi E, Sokhan A, Soltani S, Sorensen RJD, Soriano JB, Sorrie MB, Soyiri IN, Sreeramareddy CT, Stanaway JD, Stark BA, Ştefan SC, Stein C, Steiner C, Steiner TJ, Stokes MA, Stovner LJ, Stubbs JL, Sudaryanto A, Sufiyan MB, Sulo G, Sultan I, Sykes BL, Sylte DO, Szócska M, Tabarés-Seisdedos R, Tabb KM, Tadakamadla SK, Taherkhani A, Tajdini M, Takahashi K, Taveira N, Teagle WL, Teame H, Tehrani- B, Teklehaimanot BF, Terrason S, Tessema ZT, Thankappan KR, Thomson AM, Tohidinik HR, Tonelli M, Topor-Madry R, Torre AE, Touvier M, Tovani-Palone MRR, Tran BX, Travillian R, Troeger CE, Truelsen TC, Tsai AC, Tsatsakis A, Car LT, Tyrovolas S, Uddin R, Ullah S, Undurraga EA, Unnikrishnan B, Vacante M, Vakilian A, Valdez PR, Varughese S, Vasankari TJ, Vasseghian Y, Venketasubramanian N, Violante FS, Vlassov V, Vollset SE, Vongpradith A, Vukovic A, Vukovic R, Waheed Y, Walters MK, Wang J, Wang Y, Wang Y-P, Ward JL, Watson A, Wei J, Weintraub RG, Weiss DJ, Weiss J, Westerman R, Whisnant JL, Whiteford HA, Wiangkham T, Wiens KE, Wijeratne T, Wilner LB, Wilson S, Wojtyniak B, Wolfe CDA, Wool EE, Wu A-M, Hanson SW, Wunrow HY, Xu G, Xu R, Yadgir S, Jabbari SHY, Yamagishi K, Yaminfirooz M, Yano Y, Yaya S, Yazdi-Feyzabadi V, Yearwood JA, Yeheyis TY, Yeshitila YG, Yip P, Yonemoto N, Yoon S-J, Lebni JY, Younis MZ, Younker TP, Yousefi Z, Yousefifard M, Yousefinezhadi T, Yousuf AY, Yu C, Yusefzadeh H, Moghadam TZ, Zaki L, Zaman SB, Zamani M, Zamanian M, Zandian H, Zangeneh A, Zastrozhin MS, Zewdie KA, Zhang Y, Zhang Z-J, Zhao JT, Zhao Y, Zheng P, Zhou M, Ziapour A, Zimsen SRM, Naghavi M, Murray CJL. 2020. Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the global burden of disease study 2019. Lancet 396:1204–1222. https://doi.org/10.1016/S0140-6736(20)30925-9

2. Ahmed S, Shapiro NL, Bhattacharyya N. 2014. Incremental health care utilization and costs for acute otitis media in children. Laryngoscope 124:301–305. https://doi.org/10.1002/lary.24190
3. Kaur R, Morris M, Pichichero ME. 2017. Epidemiology of acute otitis media in the postpneumococcal conjugate vaccine era. Pediatrics 140:e20170181. https://doi.org/10.1542/peds.2017-0181
4. Bosch A, van Houten MA, Bruin JP, Wijmenga-Monsuur AJ, Trzciński K, Bogaert D, Rots NY, Sanders EAM. 2016. Nasopharyngeal carriage of *Streptococcus pneumoniae* and other bacteria in the 7th year after implementation of the Pneumococcal conjugate vaccine in the Netherlands. Vaccine 34:531–539. https://doi.org/10.1016/j.vaccine.2015.11.060
5. Murphy TF, Parameswaran GI, Goldstein EJC. 2009. *Moraxella catarrhalis*, a human respiratory tract pathogen. Clin Infect Dis 49:124–131. https://doi.org/10.1086/599375
6. Andreas S, Testa M, Boyer L, Brusselle G, Janssens W, Kerwin E, Papi A, Pek B, Puente-Maestu L, Saralaya D, Watz H, Wilkinson TMA, Casula D, Di Maro G, Lattanzi M, Moraschini L, Schoonbroodt S, Tasciotti A, Arora AK, Maltais F, NTHi-Mcat-002 study group. 2022. Non-typeable *Haemophilus*

influenzae-Moraxella catarrhalis vaccine for the prevention of exacerbations in chronic obstructive pulmonary disease: a multicentre, randomised, placebo-controlled, observer-blinded, proof-of-concept, phase 2B trial. Lancet Respir Med 10:435–446. https://doi.org/10.1016/S2213-2600(21)00502-6
7. Perez AC, Murphy TF. 2017. A Moraxella catarrhalis vaccine to protect against otitis media and exacerbations of COPD: an update on current progress and challenges. Hum Vaccin Immunother 13:2322–2331. https://doi.org/10.1080/21645515.2017.1356951
8. Stubbendieck RM, Dissanayake E, Burnham PM, Zelasko SE, Temkin MI, Wisdorf SS, Vrtis RF, Gern JE, Currie CR. 2023. *Rothia* from the human nose inhibit *Moraxella catarrhalis* colonization with a secreted peptidoglycan endopeptidase. mBio 14:e0046423. https://doi.org/10.1128/mbio.00464-23
9. Atlanta GA, Centers for Disease Control and Prevention. 2019. Antibiotic resistance threats in the United States, 2019. U.S Depeartment of Health and Human Services, CDC.
10. Sader HS, Mendes RE, Le J, Denys G, Flamm RK, Jones RN. 2019. Antimicrobial susceptibility of *Streptococcus pneumoniae* from North America, Europe, Latin America, and the Asia-Pacific region: results from 20 years of the SENTRY antimicrobial surveillance program (1997-2016). Open Forum Infect Dis 6:S14–S23. https://doi.org/10.1093/ofid/ofy263
11. Su P-Y, Huang A-H, Lai C-H, Lin H-F, Lin T-M, Ho C-H. 2020. Extensively drug-resistant *Haemophilus influenzae* - emergence, epidemiology, risk factors, and regimen. BMC Microbiol 20:102. https://doi.org/10.1186/s12866-020-01785-9
12. Yamada S, Seyama S, Wajima T, Yuzawa Y, Saito M, Tanaka E, Noguchi N. 2020. β-lactamase-non-producing ampicillin-resistant *Haemophilus influenzae* is acquiring multidrug resistance. J Infect Public Health 13:497–501. https://doi.org/10.1016/j.jiph.2019.11.003
13. Murray CJL, Ikuta KS, Sharara F, Swetschinski L, Robles Aguilar G, Gray A, Han C, Bisignano C, Rao P, Wool E, Johnson SC, Browne AJ, Chipeta MG, Fell F, Hackett S, Haines-Woodhouse G, Kashef Hamadani BH, Kumaran EAP, McManigal B, Achalapong S, Agarwal R, Akech S, Albertson S, Amuasi J, Andrews J, Aravkin A, Ashley E, Babin F-X, Bailey F, Baker S, Basnyat B, Bekker A, Bender R, Berkley JA, Bethou A, Bielicki J, Boonkasidecha S, Bukosia J, Carvalheiro C, Castañeda-Orjuela C, Chansamouth V, Chaurasia S, Chiurchiù S, Chowdhury F, Clotaire Donatien R, Cook AJ, Cooper B, Cressey TR, Criollo-Mora E, Cunningham M, Darboe S, Day NPJ, De Luca M, Dokova K, Dramowski A, Dunachie SJ, Duong Bich T, Eckmanns T, Eibach D, Emami A, Feasey N, Fisher-Pearson N, Forrest K, Garcia C, Garrett D, Gastmeier P, Giref AZ, Greer RC, Gupta V, Haller S, Haselbeck A, Hay SI, Holm M, Hopkins S, Hsia Y, Iregbu KC, Jacobs J, Jarovsky D, Javanmardi F, Jenney AWJ, Khorana M, Khusuwan S, Kissoon N, Kobeissi E, Kostyanev T, Krapp F, Krumkamp R, Kumar A, Kyu HH, Lim C, Lim K, Limmathurotsakul D, Loftus MJ, Lunn M, Ma J, Manoharan A, Marks F, May J, Mayxay M, Mturi N, Munera-Huertas T, Musicha P, Musila LA, Mussi-Pinhata MM, Naidu RN, Nakamura T, Nanavati R, Nangia S, Newton P, Ngoun C, Novotney A, Nwakanma D, Obiero CW, Ochoa TJ, Olivas-Martinez A, Olliaro P, Ooko E, Ortiz-Brizuela E, Ounchanum P, Pak GD, Paredes JL, Peleg AY, Perrone C, Phe T, Phommasone K, Plakkal N, Ponce-de-Leon A, Raad M, Ramdin T, Rattanavong S, Riddell A, Roberts T, Robotham JV, Roca A, Rosenthal VD, Rudd KE, Russell N, Sader HS, Saengchan W, Schnall J, Scott JAG, Seekaew S, Sharland M, Shivamallappa M, Sifuentes-Osornio J, Simpson AJ, Steenkeste N, Stewardson AJ, Stoeva T, Tasak N, Thaiprakong A, Thwaites G, Tigoi C, Turner C, Turner P, van Doorn HR, Velaphi S, Vongpradith A, Vongsouvath M, Vu H, Walsh T, Walson JL, Waner S, Wangrangsimakul T, Wannapinij P, Wozniak T, Young Sharma TEMW, Yu KC, Zheng P, Sartorius B, Lopez AD, Stergachis A, Moore C, Dolecek C, Naghavi M. 2022. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. The Lancet 399:629–655. https://doi.org/10.1016/S0140-6736(21)02724-0
14. Jim O. 2016. Tackling drug-resistant infections globally: final report and recommendations
15. Coates A, Hu Y, Bax R, Page C. 2002. The future challenges facing the development of new antimicrobial drugs. Nat Rev Drug Discov 1:895–910. https://doi.org/10.1038/nrd940
16. 2021 Antibacterial agents in clinical and preclinical development: an overview and analysis. Available from: https://www.who.int/publications-detail-redirect/9789240047655. Retrieved 22 Apr 2023. Accessed April 22, 2023
17. Jörissen J, van den Broek MFL, De Boeck I, Van Beeck W, Wittouck S, Boudewyns A, Van de Heyning P, Topsakal V, Van Rompaey V, Wouters I, Van Heirstraeten L, Van Damme P, Malhotra-Kumar S, Theeten H, Vanderveken OM, Lebeer S. 2021. Case-control microbiome study of chronic otitis media with effusion in children points at *Streptococcus salivarius* as a pathobiont-inhibiting species. mSystems 6:e00056-21. https://doi.org/10.1128/mSystems.00056-21
18. Kelly MS, Plunkett C, Yu Y, Aquino JN, Patel SM, Hurst JH, Young RR, Smieja M, Steenhoff AP, Arscott-Mills T, Feemster KA, Boiditswe S, Leburu T, Mazhani T, Patel MZ, Rawls JF, Jawahar J, Shah SS, Polage CR, Cunningham CK, Seed PC. 2022. Non-diphtheriae *Corynebacterium* species are associated with decreased risk of pneumococcal colonization during infancy. ISME J 16:655–665. https://doi.org/10.1038/s41396-021-01108-4
19. Deasy AM, Guccione E, Dale AP, Andrews N, Evans CM, Bennett JS, Bratcher HB, Maiden MCJ, Gorringe AR, Read RC. 2015. Nasal inoculation of the commensal *Neisseria lactamica* inhibits carriage of *Neisseria meningitidis* by young adults: a controlled human infection study. Clin Infect Dis 60:1512–1520. https://doi.org/10.1093/cid/civ098
20. Uehara Y, Nakama H, Agematsu K, Uchida M, Kawakami Y, Abdul Fattah AS, Maruchi N. 2000. Bacterial interference among nasal inhabitants: eradication of *Staphylococcus aureus* from nasal cavities by artificial implantation of *Corynebacterium sp*. J Hosp Infect 44:127–133. https://doi.org/10.1053/jhin.1999.0680