during mealtimes created too much of a distraction for residents and interfered with dietary care. DISCUSSION/SIGNIFICANCE OF IMPACT: It is clear from both the staff interviews and direct observations of musical activities that music is important to consider for people living with dementia in care communities. Guidelines for implementation and minimum standards would be helpful to ensure all care community residents can experience benefits highlighted by staff in this study.

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Sh-oligopeptide-72 ameliorates the proliferative defects of aging keratinocytes

Brook Abegaze[1], Richard Parenteau[1], Richard Kim[1], Giselle Vitcov[2], Victoire Gouirand[1], Merisa Piper[1] and Ruby Ghadially[1]
[1]UCSF and [2]University of Arizona

OBJECTIVES/GOALS: Aged keratinocytes are less proliferative than adult, and aged skin heals more slowly. We examined the proliferation kinetics of aged and adult human keratinocytes. We then tested whether an extrinsic agent, sh-oligopeptide-72, can ameliorate these defects. METHODS/STUDY POPULATION: We used live cell imaging (LCI) to examine the proliferation kinetics of aged (73–92y) and adult (34–49y) passage zero human keratinocytes. We then incubated aged keratinocytes with a peptide, sh-oligopeptide-72 (purported to improve keratinocyte proliferation), or vehicle (PBS). Lineage trees of cell divisions were constructed to determine cell cycle duration and the proliferation/differentiation outcomes of each division. To assess wound healing, cells were isolated from 3 patients, 82–92y, and plated in 2-well culture dishes with inserts. Wells were treated with sh-oligopeptide-72 (100 ng/ml) or vehicle (PBS). At confluence, the insert was removed leaving a well-defined 500 μm gap. The time until 100% closure of the defect was obtained using LCI and the wound healing size tool. RESULTS/ANTICIPATED RESULTS: There was no significant difference in the number of stem cell (SC) colonies between aged and adult keratinocytes. However, aged keratinocytes produced more aged committed progenitor (CP) colonies ($P<0.0001$). Adult CP, but not stem, colonies were significantly larger than aged ($P = 0.0001$), and this was associated with earlier terminal differentiation ($P = 0.0005$). Aged SC and CP colonies exhibited a higher proportion of differentiation divisions, and their cell cycle duration (CCD) was increased. Sh-oligopeptide-72 rescued the increased terminal differentiation as well as decreased the CCD in SC colonies. Sh-oligopeptide decreased the mean closure time of the wound assays (143h vs. 204h, $P = 0.04$). DISCUSSION/SIGNIFICANCE OF IMPACT: Sh-oligopeptide-72 reversed many of the proliferation defects that develop in aged SC colonies. Wound assays show that this results in improved keratinocyte function. These results suggest that the age-related changes in growth dynamics can be modified in response to extrinsic signals in vitro.

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Phage Wars: Uncovering the resistance strategies of *Escherichia coli* O157:H7*

Edwin Rivera-Lope, Rivera-Lopez Edwin Omar, Moriniere Lucas, Kazakov Alexey, Deutschbauer Adam, Arkin Adam, Mutalik Vivek and Dudley G. Edward
PennState University

OBJECTIVES/GOALS: The goal of this work is to understand the physiological profile of phage susceptibility and identify candidate phage defense mechanisms. Additionally, it aims to determine the host receptors targeted by bacteriophages to infect E. coli O157:H7 through random bar code transposon-site sequencing (RB-TnSeq). METHODS/STUDY POPULATION: A collection of 109 E. coli O157:H7 strains from environmental, food, and animal sources were analyzed, representing phylogenetic lineages corresponding to clades 2, 3, 5, 6, 7, and 8. Phage susceptibility profiles were determined using 23 bacteriophages, assessing plaque morphology. Using the O157:H7 genomes, a genomic analysis was conducted with the Prokaryotic Antiviral Defense Locator (PADLOC), which identified putative phage defense systems through sequence homology. Additionally, 5 RB-TnSeq libraries were generated in representative strains to study loss-of-function mutations. These libraries will be screened against a subset of diverse phage to identify the receptors involved in phage adsorption. RESULTS/ANTICIPATED RESULTS: The phage resistance patterns showed susceptibility varied across clades, suggesting distinct mechanisms. Several defense systems were identified using PADLOC, including restriction-modification, Cas, Lamassu, and Druantia. Phage defense candidate (PDC) systems were identified, showing homology to known systems, though their specific function remains unknown. Clade 7.2 exhibited higher phage resistance and a greater presence of PDCs compared to the other clades. Five saturated RB-TnSeq libraries were constructed in O157:H7, achieving 84.5–89% gene coverage. These libraries will facilitate the identification of receptors involved in phage adsorption and resistance. DISCUSSION/SIGNIFICANCE OF IMPACT: This study deepens our understanding of phage resistance in E. coli O157:H7 by identifying key defense systems and receptors. The discovery of novel antiviral mechanisms offers promising targets for phage-based interventions, potentially enhancing strategies for controlling this dangerous pathogen.

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Genetic heterogeneity and antifungal resistance within Candida infecting populations*

Elizabeth Wash, Christopher Zajac and Anna Selmecki
University of Minnesota

OBJECTIVES/GOALS: This study will assess population heterogeneity in Candida bloodstream infections by quantifying antifungal resistance, fitness, and genomic diversity to understand clonality and develop a high-throughput screening tool to detect population-level resistance to update clinical practice. METHODS/STUDY POPULATION: This study assesses antifungal resistance and population heterogeneity in Candida bloodstream isolates collected through multiple Midwest hospitals. Blood samples are plated to isolate single colonies and population samples, which are then archived. We test resistance to key antifungals using EUCAST guidelines, conduct growth curve assays, and perform whole-genome sequencing to determine genetic diversity. A high-throughput screening method tracks colony growth under different drug conditions using time-lapse imaging and custom analysis software. The findings will reveal the extent of antifungal resistance and genetic variation within infecting populations, informing better clinical management. RESULTS/ANTICIPATED RESULTS: Preliminary analysis of *Candida glabrata* bloodstream isolates show significant heterogeneity in colony morphology, antifungal resistance, and fitness. Some single colonies exhibit higher minimum inhibitory concentration values for micafungin and fluconazole than the overall population, while others show reduced susceptibility to amphotericin B, highlighting diverse resistance profiles. Growth assays reveal distinct

https://doi.org/10.1017/cts.2024.1017

fitness phenotypes within these populations. This variation underscores the limitations of single-colony testing and suggests a need for population-level resistance screening. We anticipate genomic analyses will identify genetic diversity underlying these differences, supporting a more comprehensive clinical approach to treatment. DISCUSSION/SIGNIFICANCE OF IMPACT: This study reveals notable intrapopulation heterogeneity in *Candida glabrata*, including variations in colony morphology, antifungal resistance, and fitness. Findings highlight genomic diversity, introduce a novel screening method for resistance, and emphasize the need for population-level testing in clinical practice.

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Quality of communication with parents of critically ill infants

Katherine Guttmann and Cardinale Smith
Icahn School of Medicine at Mount Sinai

OBJECTIVES/GOALS: Communication between clinicians and parents of seriously ill infants is understudied. This study aims to 1) define high-quality communication with parents of critically ill infants and 2) evaluate the psychometric properties and validity of a measure of high-quality communication in parents of critically ill infants. METHODS/STUDY POPULATION: 1) Using participant observation and semi-structured interviews of 35 parents of hospitalized infants, I will conduct content analysis to describe high-quality prognostic communication with parents of infants in the pediatric intensive care unit. Using descriptions captured during participant observation and in semi-structured interviews, I will produce a novel definition of high-quality communication with parents of seriously ill infants. I will also explore parent experiences of communication by race. 2) I will validate a measure of communication quality in parents of 200 neonatal and pediatric intensive care unit patients. I will use factor analysis to evaluate the extent to which responses map onto an established construct and assess dimensionality and reliability. RESULTS/ANTICIPATED RESULTS: 1) I anticipate finding that identification of high-quality communication will be consistent between participant observation and interviews and will track with Wreesmann's framework. I hypothesize that minoritized parents are more likely to receive low-quality communication. 2) I hypothesize that the measure of communication quality will be valid and reliable in the neonatal and pediatric intensive care units. DISCUSSION/SIGNIFICANCE OF IMPACT: I will explore communication quality in a novel setting for which limited data are currently available, establishing a measure for future pediatric communication research and identifying targets for interventions to improve communication quality. Better understanding of communication with parents of sick infants will lead to improved outcomes.

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Social cognition is associated with social problems in adolescents with Tourette syndrome

Kelly Watson, Brandon Low, Abigail L.B. Snow, Isabelle Taylor and David A. Isaacs
Vanderbilt University Medical Center

OBJECTIVES/GOALS: To assess theory of mind and empathy in adolescents with Tourette syndrome (TS) and examine their association with social problems. This study aims to extend research in social cognition to an adolescent cohort with TS and identify a potential modifiable risk factor for social problems in TS that may serve as a novel intervention target. METHODS/STUDY POPULATION: We will enroll 50 adolescents with TS (ages 11–17) and 50 demographically matched controls along with one parent to complete a single in-person study visit. Adolescents with TS will be recruited through the Vanderbilt Center for TS and other Tic disorders. Controls will be recruited using university listservs and flyers posted in community and primary care settings. Adolescents will complete the NEPSY-II to assess theory of mind abilities and the Multifaceted Empathy Test – Juvenile to assess empathy with negative emotions. Parents will complete the Child Behavior Checklist to assess adolescent social problems. RESULTS/ANTICIPATED RESULTS: Based on evidence of low self-other distinction in TS, we hypothesize TS adolescents will make more errors about the mental states of others (theory of mind) and report greater emotional reactions to faces (empathy) compared to controls. Further, greater social problems will be associated with greater disturbances in social cognition. To date, 15 adolescents with TS and 15 matched controls have completed the assessment (67% male; Mage = 14.33 in both groups). Within this sample, adolescents with TS experienced more social problems than controls (Cohen's $d = .74$, $p = .03$). There were no between-group differences in theory of mind or empathy in this pilot sample. However, higher levels of both theory of mind and empathy were linked to experiencing greater social problems in the TS sample only (p's $< .05$). DISCUSSION/SIGNIFICANCE OF IMPACT: Preliminary findings suggest that while social cognition did not differ between groups, TS adolescents exhibiting high levels of theory of mind and empathy appear to struggle socially. This work could inform future interventions by highlighting the need to focus on social cognition and how these skills translate into social behaviors.

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Aging and sarcopenia of the diaphragm muscle

Genesis Hernandez Vizcarrondo, Matthew J. Fogarty, Humzah Abdulkader, Lucy Sieck, Carlos B. Mantilla and Gary C. Sieck
Department of Physiology and Biomedical Engineering, Mayo Clinic

OBJECTIVES/GOALS: Understand the impact of sarcopenia on the main respiratory muscle, the diaphragm (DIAm). We hypothesize that in the DIAm of older (i.e., 24 months) compared to younger (i.e., 6 months) rats, maximum specific force (P0) is reduced, maximum shortening velocity (Vmax) is slower, maximum power output is reduced, and endurance is improved. METHODS/STUDY POPULATION: Mid-costal DIAm strips were excised from 6-month (n = 8; 4 female and 4 male) and 24-month (n = 8; 4 female and 4 male) rats. The DIAm was stimulated using platinum plate electrodes, and mechanical and endurance properties were measured (at 26oC). RESULTS/ANTICIPATED RESULTS: In the DIAm, maximum tetanic formce (P0) decreased by ~35%, maximum velocity of shortening (Vmax) slowed by ~20%, and peak power output was reduced by ~35% in 24-month compared to 6-month rats. During repetitive isovelocity (30% Vmax; approximating peak power output) contractions, endurance (the period during which power output was sustained) of the DIAm was unaffected by aging. Corresponding with previous findings, DISCUSSION/SIGNIFICANCE OF IMPACT: The changes in DIAm mechanical

https://doi.org/10.1017/cts.2024.1017