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

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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*

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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*

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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

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