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Adjuvants for the potentiation of the activity of β -lactam antibiotics against methicillin-resistant *Staphylococcus aureus**

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OBJECTIVES/GOALS: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a human bacterial pathogen and is classified as a serious threat. MRSA has become resistant to most B-lactam antibiotics (penicillins and cephalosporins). The goal of this study is to identify an antibiotic adjuvant capable of resurrecting B-lactams for the treatment of MRSA infections. **METHODS/STUDY POPULATION:** A fluorescence-reporter assay was used to screen a compound library. Minimum-inhibitory concentrations were assessed for the compounds against various MSSA and MRSA strains. A common resistance mechanism to B-lactams by MRSA is by the function of the bla operon. One gene in this operon encodes for a B-lactam sensor/signal transducer protein BlaR, the primary target of this study. Inhibition of BlaR by compound 1 (best potentiator of oxacillin) was studied by nano-differential scanning fluorimetry (nanoDSF), surface plasmon resonance (SPR), scanning electron microscopy (SEM), and time-kill assays. **RESULTS/ANTICIPATED RESULTS:** We identified 80 compound hits from a 1,974-compound NCI library. Twenty-four compounds showed potentiating ability (2- to 4,096-fold decrease in MIC for oxacillin). Seven compounds exhibited melting temperature shifts by nanoDSF of BlaR, indicating binding. SPR determined compound 1 has a binding affinity of 31 micromolar to BlaR-SD. SEM images showed disruption in the *S. aureus* cell wall on exposure to compound 1 and oxacillin. *S. aureus* N315 showed 3-log reduction in bacterial count treated with a mixture of compound 1 and oxacillin. **DISCUSSION/SIGNIFICANCE OF IMPACT:** Compound 1 targets BlaR-SD, which restores *S. aureus* susceptibility to treatment by oxacillin. There are currently few antibiotics available in the clinic capable of treating MRSA infections. The combination hold promise of a treatment option for MRSA.

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Inclusion of cytomegalovirus viral Fc gamma receptors in a glycoprotein B protein subunit vaccine improves Fc-mediated effector responses

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OBJECTIVES/GOALS: We hypothesized that adding cytomegalovirus (CMV) viral Fc gamma receptors (vFcγRs) to a glycoprotein B (gB) protein subunit vaccine would improve vaccine-elicited Fc mediated effector functions such as antibody dependent cellular phagocytosis (ADCP) and cytotoxicity (ADCC), over gB subunit alone. **METHODS/STUDY POPULATION:** We immunized rabbits (n = 4 per group) at Weeks 0, 4, and 8 with 20μg gB alone or with one vFcγR (gp34, gp68, or gp95) at 20μg or 40μg, adjuvanted with squalene emulsion, Addavax. Plasma from immunized rabbits was analyzed for antigen-specific IgG binding via enzyme-linked immunosorbent assays (ELISAs). ADCP was measured by

conjugating whole virions to a fluorescent marker (AF647), incubating the fluorescent virus with rabbit plasma, and measuring uptake of virus by THP-1 monocytes via flow cytometry. ADCC was measured by natural killer cell degranulation via flow cytometric detection of CD107a expression following co-incubation with CMV-infected fibroblasts and rabbit plasma. **RESULTS/ANTICIPATED RESULTS:** Each vFcγR demonstrated immunogenicity, although average vFcγR-binding IgG titers were between 4- to 10-fold higher in animals receiving the 40μg dose of each vFcγR compared to the 20μg dose. We observed similar IgG binding responses against gB among all vaccine groups. Comparing groups at peak immunogenicity (Week 10), ADCP responses were improved over gB alone by approximately twofold in animals receiving 40μg of each vFcγR. This effect was maintained across several human CMV strains with variable vFcγR genes. ADCC responses were undetectable in all animals immunized with gB alone, yet those receiving 40μg gp34 or gp95 demonstrated detectable ADCC. **DISCUSSION/SIGNIFICANCE OF IMPACT:** HCMV-specific ADCP and ADCC are associated with protection against vertical CMV transmission, so a vaccine including vFcγRs which can improve vaccine-elicited Fc-effector responses is promising toward reducing the immense global impact of congenital CMV and associated neurologic birth defects.

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Music use for dementia care in urban elder care communities in Northeast Kansas

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OBJECTIVES/GOALS: Research supports the use of music to improve the care and well-being of adults living with dementia; however, the practice and implementation of music in elder care communities is not regulated. The goal of this qualitative study was to survey elder care communities in Northeast Kansas to determine the use of music with people living with dementia. **METHODS/STUDY POPULATION:** We interviewed staff (n = 10) at five elder care communities in the Kansas City Metro area and observed musical activities and artifacts in shared living spaces within each community. Interview questions included details of the frequency and purpose of using music, who determined which music to use, and any effects, positive or negative, the interviewee believed to be associated with the use of music. Musical events, visiting musicians or music therapists leading group sing-alongs were observed at two communities, and music-related activities led by staff were observed at two others. **RESULTS/ANTICIPATED RESULTS:** Music was used in some way at each of the five communities. Each location had recorded music available to residents in the shared living spaces, and most had a piano in the main lounge area. During the sing-along and music-related activities, residents were observed singing along to songs from memory, engaging with one another and the group leader and smiling. Staff employed by each community varied in their level of musical training and experience, from none to a full-time music therapist in residence. Staff interviewed said they believed music was helpful to aid memory recall, reduce anxiety, and to engage interest. Interestingly, a music therapist at one site also described how music

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