Methods. In this case series, we evaluated the impact of mNGS on the clinical management of IE by conducting a retrospective chart review of children hospitalized with IE from January 1, 2017 to January 17, 2020. Inclusion criteria included subjects 0–21 years of age who had a diagnosis of IE based on admission diagnosis and problem list. Demographics, echocardiography and diagnostics were obtained by chart review.

Results. We identified 14 children who were diagnosed with IE, 10 of whom had mNGS sent for diagnostic testing. mNGS detected an organism in 8 of 10 (80%) children as compared to standard of care blood cultures and 16S PCR which identified an organism in only 5 of 10 (30%). Out of 8 subjects with organisms identified by mNGS, clinical management was impacted in 4 of 8 subjects and 3 subjects were only diagnosed through mNGS.

Conclusion. Although blood and tissue cultures are the historical gold standard for organism identification in IE, new diagnostic modalities such as mNGS may be more sensitive. This could lead to more targeted and possibly more effective therapeutic regimens in cases when empiric antibiotic therapy may hinder pathogen identification. In at least one instance, a change in antibiotic choice to penicillin to target *Corynebacterium diphtheriae* resulted in a clinical cure that may not have otherwise been achieved. As a result of mNGS, three other subjects also had changes in management that resulted in narrowing of antibiotic choice or adjustments to the antibiotic regimen. Our observations suggest that mNGS is more sensitive than standard blood cultures and that the clinical utility of mNGS may be beneficial to the management of infective endocarditis in children.

#4: Antibiotic Durations for Skin and Soft Tissue Infections in Pediatric Urgent Care Clinics

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Background. Skin and soft tissue infections (SSTIs) are the second most common diagnosis leading to pediatric antibiotic prescriptions in the outpatient setting after respiratory diagnoses. However, most antibiotic stewardship programs have mainly focused on the latter. Children seen in the ambulatory setting for SSTIs often receive >7 days of antibiotics, although current society guidelines recommend 5–7 days for most diagnoses.

Objectives. To determine the baseline percentage of patients receiving antibiotic prescriptions for >7 days for SSTIs in urgent care clinics (UCC)s of a pediatric health system and to evaluate factors that influence providers towards longer durations.

Methods. We built a report that extracted patient encounters from the three UCCs based on International Classification of Diseases (ICD)-10 codes for common SSTIs including impetigo, abscesses, cellulitis, erysipelas, folliculitis, paronychia, and animal bites. Data was pulled from June 2019 through June 2020. The report included patient age, concomitant diagnoses, antibiotics prescribed and their duration. We excluded encounters if the patient was transferred to the emergency department or admitted, the patient was younger than 3 months of age, no antibiotics were prescribed, or if there was a concurrent infectious diagnosis affecting antibiotic duration. We sent a 22-question survey to UCC providers to understand prescribing habits particularly focusing on factors prompting administration of longer antibiotic courses.

Findings. From June 2019-June 2020, we reviewed 2,575 encounters; we excluded 208 of those (8%). 823 (35%) of patients received >7 days of antibiotics for SSTIs while 1181 (50%) received 5–7 days and 35 (1%) received <5 days of antibiotics. 328 (14%) received topical therapy only. Most common antibiotics prescribed included cephalexin, clindamycin, and trimethoprim-sulfamethoxazole. A mild improvement in the 5–7 days duration was noted through our study period (Figure 1). The survey was sent to 50 providers with 27 responding (54% response rate). Of providers surveyed, 5 (19%), 7 (26%), and 8 (29%), expressed being uncomfortable with a 5-day treatment course for cellulitis, erysipelas, and abscesses respectively. Barriers for shorter treatment courses included concern for acute rheumatic fever development, parental pressure, fear of complications, and accustomed antibiotic duration.

Conclusion. A third of children with SSTIs in our UCCs receive long courses of antibiotics. A mild improvement noted in our study period may be due to existing antibiotic stewardship interventions. Specific provider concerns leading to overprescribing will be targeted by quality improvement efforts.

a

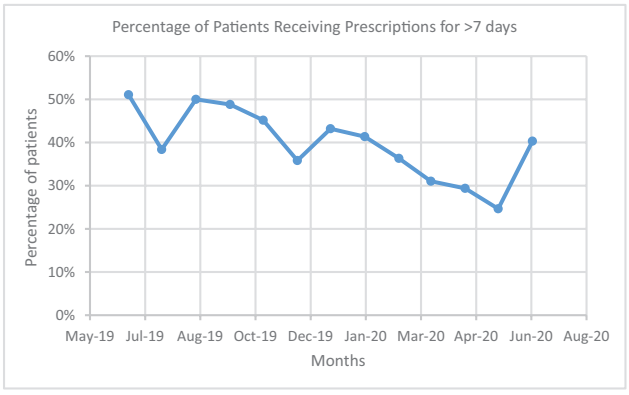

Figure 1a: Run Chart Showing the Percentage of Patients Diagnosed with Skin and Soft Tissue Infections Receiving Over 7 days of Oral Antibiotic Therapy

b

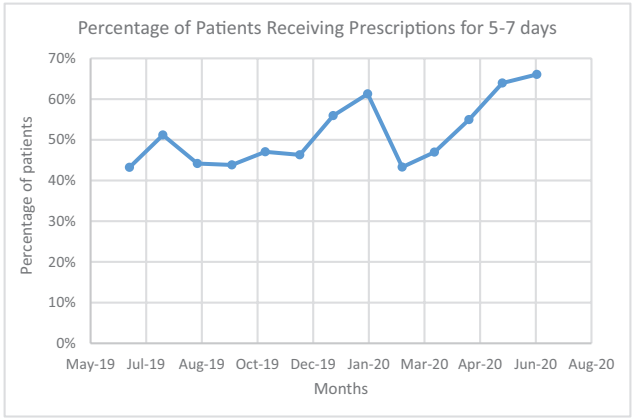

Figure 1b: Run Chart Showing the Percentage of Patients Diagnosed with Skin and Soft Tissue Infections Receiving 5 to 7 days of Oral Antibiotic Therapy

#8: Microbiome and immune disruption accompany mouse death in a gnotobiotic mouse model of neonatal sepsis

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Background. Premature infants frequently receive antibiotics, which diminishes gut microbial diversity and increases susceptibility to infections by antibiotic resistant pathogens. Neonates with decreased gut microbiota diversity, termed dysbiotic, have dysregulated immune systems marked by increased concentrations of circulating activated T cells and decreased concentrations of circulating neutrophils and dendritic cells. We hypothesize that antibiotics (1) enrich for pathogens within the gut, 2) promote a systemic, proinflammatory host response, and 3) cause death in an antibiotic- and microbiome-specific manner in a gnotobiotic model of preterm gut microbiota disruption.

Methods. We colonized germ free (GF) dams with stools from preterm infants. Mouse pups acquire this neonatal microbiota, and at 10 days of life (DOL), we treat them with clinically-relevant doses of antibiotics subcutaneously for 3 days. We determined serum concentrations of antibiotics in 10 DOL pups using tandem mass spectrometry to achieve approximate pharmacokinetics as observed in the neonatal intensive care unit (NICU). We ascertained phylogenetic composition using metagenomic shotgun sequencing of individual pup fecal samples longitudinally. We performed flow cytometry on peripheral blood and gut permeability assays to determine the local and peripheral immune response.

Results. We found adding probenecid prolonged the half-life of ampicillin and meropenem allowing for an approximation of serum levels observed in the NICU with an every 8 hour dosing regimen. Using two representative microbiomes from human neonates (hereafter referred to as microbiota A or B), we show that 95% of pups given microbiota A survive versus 54% given microbiota B after meropenem/probenecid treatment (Fig. 1A; $p<0.01$; n= 18–42 mice in 3–6 independent experiments). Conversely, only 28% of microbiota-A humanized pups survive during ampicillin/probenecid treatment (Fig. 1; $p<0.0001$). Ampicillin-resistant *Klebsiella* species and *E. coli* dominated the gut of microbiota A-humanized pups who succumbed during ampicillin/probenecid treatment whereas *Enterococci* dominated the gut of microbiota B-humanized pups who died during treatment. To test the reproducibility of this phenotype, we colonized mice with 2 additional preterm neonatal microbiomes with similar compositions to microbiota A and B (D and C, respectively). We found that microbiota-C humanized pups were similarly dominated by *Enterococcus faecalis* resulting in 42% mortality during meropenem/probenecid treatment (Fig. 1). Pups colonized with microbiota B had decreased circulating granulocytes, B cells, and CD8+ T cells at sacrifice after treatment compared to microbiota A-humanized pups. We next assessed gut permeability after antibiotic treatment by measuring 4kDa FITC-Dextran in mouse serum after oral gavage. Microbiota-A humanized pups treated with ampicillin/probenecid and microbiota B-humanized pups treated with meropenem/probenecid had elevated serum levels of FITC-Dextran ($p<0.05$ relative to vehicle control, one way ANOVA), indicative of increased gut permeability.

Conclusions. Our model of preterm microbiota perturbation by antibiotics demonstrates increased gut permeability, proinflammatory immune response, and death dependent on the microbiota-antibiotic combination. Our transgenerational humanized-microbiota mouse model can be utilized to determine antibiotic by microbiota perturbation and examine risks of late onset sepsis from specific antimicrobial administration. This research can lead to a personalized medicine approach of antibiotic treatment in the NICU to limit antibiotic side effects and mortality.

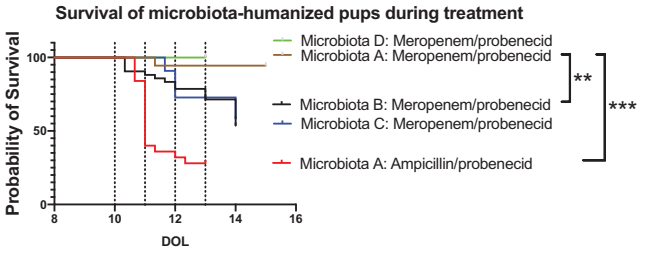

Figure 1: Microbiota-humanized pups die during specific antibiotic treatment. P values assessed with Log-Rank test. **, p<0.01; ***, p<0.0005

#10: Tropism, Susceptibility, and Infectivity of Differentiated Human Tonsillar Epithelial Cells by Different Influenza Viruses

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Introduction. Influenza viruses cause significant socioeconomic impact due to annual outbreaks and pandemic risks. Human tonsil epithelium cells (HTEC) are a heterogeneous group of actively differentiating epithelia comprising stratified squamous epithelium and reticulated crypt cells with abundant keratin expression.

Hypothesis. We hypothesized that the tonsils are a primary site for influenza infection and sustained viral replication.

Methods and Results. Primary HTEC (ScienCell Research Laboratories) were grown using an air-liquid interface and infected apically with different influenza viruses at various MOIs to measure viral growth kinetics. These cells were highly differentiated, with subpopulations of cells including ciliated, non-ciliated cells and specialized cells with secretory functions. There was a heterogenous distribution of both human-like (α2,6-linked) and avian-like (α2,3-linked) sialic acid receptors. The HTEC surface and crypts were lined with pseudostratified columnar ciliated cells possessing both α2,6-linked and α2,3-linked sialic acid receptors that were interrupted by patches of reticular epithelial cells. The HTEC epithelial cells were permissive for growth of influenza A and B viruses. A subset of cells, mostly ciliated cells, underwent apoptosis while others including non-ciliated cells remained intact despite being positive for influenza virus nucleoprotein. Interestingly, differences were seen between subtypes with colocalization of H3N2 virus and non-ciliated cells while H1N1 virus mostly associated with ciliated cells.

Conclusion. Our results implicated human tonsillar crypt epithelium as a site for influenza virus replication. The tonsil epithelium cell culture differentiated system provides a valuable in vitro model for studying cellular tropism, infectivity, cytokines immune response and the pathogenesis of influenza viruses for better development of effective universal vaccine and therapies against different strains of influenza viruses.

#12: The Safety and antibody kinetics of COVID-19 Convalescent Plasma for the Treatment of Moderate to Severe Cases of SARS-CoV-2 Infection in Pediatric Patients

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Introduction. A novel Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was identified as the pathogen responsible for a serious, life threatening respiratory infection (COVID-19) initially reported in Wuhan, China which rapidly spread worldwide resulting in global pandemic. Multiple immunological and pharmacological therapies have been evaluated with variable results. Convalescent plasma has been used in previous outbreaks such as Influenza 1918 and 2009, Ebola, MERS and SARS with good efficacy and safety reported. There have been multiple large reports on the safety of COVID-19 convalescent plasma (CCP) for the treatment of this serious infection in the adults; controversy has ensued regarding its efficacy. Pediatric data on CCP use are limited.

Methods. We conducted a prospective, open label treatment trial using CCP (10 ml/kg up to 1 unit) for the treatment of COVID-19 in pediatric patients with moderate to severe disease or high risk for serious illness. Safety and antibody kinetics and outcome data were collected.

Results. Eighteen moderate to severe COVID19 pediatric patients were enrolled and received CCP early in their illness (median days of symptoms XX). We observed no infusion related adverse events, no hematological or metabolic adverse events were noted during the hospitalization and at 3 weeks after infusion follow up. Sixteen patients demonstrated significant clinical improvement by day seven post infusion, as measured by the WHO eight-category ordinal severity scale for COVID-19. Presence of SARS-COV-2 anti-nucleocapsid IgG was demonstrated in all CCP specimens, a significant increase in antibodies was demonstrated in CCP recipients 24 hours after receiving CCP. Sustained high levels of anti- SARS-COV-2 anti-nucleocapsid IgG was demonstrated at 7- and 21-days post-transfusion. A transient IgM response not associated with CCP was noted. Eleven (61.1%) patients were discharge to home by day 7 post CCP infusion. One patient remained on invasive ventilatory support 21 days after CCP infusion and was eventually discharged to an intermediate care facility, one patient died retrospectively confirmed to have been brain death before receiving CCP.

Conclusion. We conclude that CCP was well tolerated in pediatric patients. CCP resulted in rapid increase of antibodies and sustained levels 21 days after infusion suggesting that CCP did not interfere with patients own immune response. More data are necessary to establish efficacy of this intervention in children, but our patients clinical course suggests benefit of CCP. Pediatric patients with moderate to severe COVID-19 may benefit of CCP when used early in the course of illness in conjunction with other immunological or antiviral interventions as needed.

#13: Respiratory and Intestinal Epithelial Cells Exhibit Differential Susceptibility and Innate Immune Responses to Contemporary EV-D68 Isolates

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Background. Enterovirus D68 (EV-D68) has been implicated in outbreaks of severe respiratory illness and associated with acute flaccid myelitis (AFM), a disease which causes paralysis in previously healthy patients, mostly children. AFM peaked in even numbered years, at least 2014–2018. While 2020 was expected to be a peak AFM year, few cases were seen, likely due to non-specific social distancing measures due to SARS-CoV-2. EV-D68 is primarily described as a respiratory pathogen, in contrast to 'classic' enteroviruses that are spread via the fecal-oral route. However, similar to other enteroviruses, EV-D68 has been detected in wastewater, suggesting it might also have an enteric route of transmission.

Methods. We used a panel of EV-D68 isolates, including a historic isolate from 2009 and multiple contemporary isolates from AFM peak years to define dynamics of viral replication and host response to infection. We performed comparative studies in primary human bronchial epithelial cells grown at an air-liquid interface and in primary human stem-cell derived intestinal enteroids. These human primary cell-based models more accurately reflect the cells targeted by EV-D68 *in vivo*. We defined growth characteristics, temperature sensitivity, infection polarity, and acid sensitivity in these parallel models. We used unbiased Luminex-based multianalyte profiling and bulk RNA-sequencing to define the innate immune response in each model.

Results:

1. Respiratory and intestinal cell lines were permissive to both historic and contemporary EV-D68 isolates, but there were isolate-specific differences in temperature sensitivity at 33°C or 37°C, with many contemporary isolates replication-competent at both temperatures.
2. Using simulated intestinal fluids, which recapitulate pH, bile acid, and phospholipid composition of the GI tract, we showed that many contemporary EV-D68 isolates are more tolerant to the conditions of the GI tract than previously recognized.
3. Primary human bronchial epithelial cells were largely resistant to EV-D68 replication, with only a single contemporary isolate of the panel able to replicate robustly. Only two isolates were able to replicate in human enteroids, both contemporary.
4. Primary human bronchial epithelial cells, but not enteroids, mount a robust innate immune response to EV-D68 infection, characterized primarily by type III interferons, and to a lesser degree, type I interferons, for historic and contemporary isolates. There were not significant differences in innate immune response at 33°C or 37°C.
5. Blockade of IFN response in human bronchial epithelial cells allows for recovery of viral replication in respiratory epithelium, supporting a mechanism for this signaling in the control of EV-D68 viral replication in the airway.

Conclusions. Our findings suggest that a subset of contemporary isolates of EV-D68 have the potential to target both the human airway and gastrointestinal tracts as a potential route of infection, identifying a previously unrecognized potential route of infection as well as defining, for the first time, the innate immune response to infection in multiple relevant primary epithelial models.

These findings are highly significant and are the first to characterize the viral replication and host innate immune response to a diverse panel of historic and contemporary EV-D68 isolates in both the respiratory and intestinal tracts.