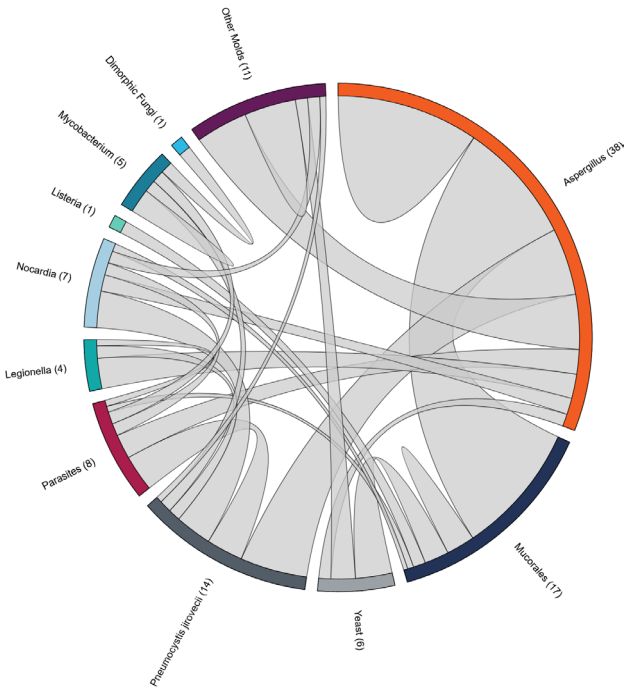
Table 1. Categorization of Co-infections with Pathogens of Critical Importance*

Pathogens	# of co-infections	Pathogens	# of co-infections	Pathogens	# of co-infections
***Aspergillus* sp.** (Asp)	**38**	***Mucorales*** (Muc)	**17**	**Parasites**	**8**
Asp + Asp (9) -*Aspergillus flavus*/*oryzae* + *Aspergillus fumigatus* -*Aspergillus niger* + *Aspergillus fumigatus* -*Aspergillus flavus*/*oryzae* + *Aspergillus nivoflumigatus* -*Aspergillus flavus*/*oryzae* + *Aspergillus tubingensis*	5 2 1 1	Asp + Muc (8)* Muc + Muc + Trich (2) -*Rhizopus microsporus* + *Rhizopus oryzae* Muc + Muc (1) -*Rhizopus microsporus* + *Rhizopus microsporus* Muc + Trich (1) -*Rhizomucor pusillus* + *Trichosporon asahii* Muc + List (1) -*Rhizomucor pusillus* + *Listeria monocytogenes* Asp + Muc + Parasite (1)* Asp + Asp + Muc (1)* Asp + Asp + Asp + Muc (1)* Asp + Muc + Muc + Noc (1)*	8 2 1 1 1 1 1	PJP + Parasite (3)* Parasite + Noc (1) -*Trichomonas vaginalis* + *Nocardia elegans* Parasite + Myco (1) -*Mycobacterium haemophilum* + *Amoeba* Asp + Muc + Parasite (1)* Asp + Leg + Parasite (1)* Asp + Muc + Parasite (1)*	3 1 1 1 1 1
Asp + Muc (8) -*Aspergillus fumigatus* + *Rhizopus microsporus* -*Aspergillus fumigatus* + *Rhizopus oryzae* -*Aspergillus fumigatus* + *Rhizomucor miehei* -*Aspergillus flavus*/*oryzae* + *Rhizopus oryzae* -*Aspergillus niger* + *Rhizopus delemar* -*Aspergillus flavus*/*oryzae* + *Rhizomucor pusillus* -*Aspergillus fumigatus* + *Rhizomucor pusillus* Asp + PJP (5) -*Aspergillus fumigatus* + *Pneumocystis jirovecii* -*Aspergillus oystoni* + *Pneumocystis jirovecii* Asp + NA/NM + NA/NM (2) -*Aspergillus chevalieri* + *Trichoderma longibrachiatum*/*atroviride* + -*Aspergillus calidoustus* + *Trichoderma longibrachiatum* -*Aspergillus calidoustus* + *Trichoderma longibrachiatum* + *Fusarium proliferatum* Asp + Leg (1) -*Aspergillus flavus*/*oryzae* + *Legionella pneumophila* Asp + Noc (1) -*Aspergillus fumigatus* + *Nocardia vinacea*/*fusca* Asp + Parasite (1) -*Aspergillus fumigatus* + *Acanthamoeba* Asp + Trich (1) -*Aspergillus fumigatus* + *Trichosporon asahii* Asp + NA/NM (1) -*Aspergillus flavus*/*oryzae* + *Alternaria alternata* Asp + Asp + NA/NM (1) -*Aspergillus subglaucus* + *Aspergillus fumigatus* + *Fusarium proliferatum* Asp + NA/NM + PJP (1) -*Aspergillus fumigatus* + *Fusarium fujikuroi* + *Pneumocystis jirovecii* Asp + Muc + Parasite (1) -*Aspergillus fumigatus* + *Lichtheimia ramosa* + *Strongyloides stercoralis* Asp + Asp + Muc (1) -*Aspergillus flavus*/*oryzae* + *Aspergillus terreus* + *Lichtheimia corymbifera* Asp + Leg + Parasite (1) -*Aspergillus fumigatus* + *Legionella feeleii*/*tunisiensis* + *Exophiala*/*salmonis* Asp + Asp + Leg (1) -*Aspergillus calidoustus* + *Aspergillus fumigatus* + *Legionella feeleii* Asp + Asp + Asp + Muc (1) -*Aspergillus fumigatus* + *Aspergillus flavus*/*oryzae* + *Aspergillus tubingensis* + *Rhizomucor pusillus* Asp + Asp + Asp + NA/NM (1) -*Aspergillus fumigatus* + *Aspergillus flavus* + *Aspergillus terreus* + *Nechia haematococca*/*cuenoi solani* Asp + Muc + Muc + Noc (1) -*Aspergillus fumigatus* + *Rhizomucor pusillus* + *Rhizopus microsporus* + *Nocardia farcinica*	38 5 2 1 1 2 1 1 1 1 1 4 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	***Pneumocystis jirovecii*** (PJP) Asp + PJP (5)* PJP + Parasite (3) -*Pneumocystis jirovecii* + *Toxoplasma gondii* -*Pneumocystis jirovecii* + *Enterocytozoon bieneusi* PJP + Noc (2) -*Pneumocystis jirovecii* + *Nocardia cyriacigeorgica* -*Pneumocystis jirovecii* + *Nocardia asiatica* PJP + Leg (1) -*Pneumocystis jirovecii* + *Legionella pneumophila* PJP + Myco (1) *Pneumocystis jirovecii* + *Mycobacterium magenticense* Asp + NA/NM + PJP (1)*	**14** 5 2 1 2 1 1 1 1	***Mycobacteria sp.*** (Myco) Myco + EM (1) -*Mycobacterium avium complex* + *Histoplasma capsulatum* Myco + Myco + Myco (1) -*Mycobacterium abscessus*/*chelonae*/*Mycobacterium mucogenicum* + *Mycobacterium bolletii*/*arenes* PJP + Myco (1)* Parasite + Myco (1)*	**4** 1 1 1 1
		***Non-Aspergillus Non-Mucorales* Molds** (NA/NM)	**10**	***Legionella sp.*** (Leg) Asp + Leg (1)* Asp + Leg + Parasite (1)* Asp + Asp + Leg (1)* PJP + Leg (1)*	**4** 1 1 1 1
		NA/NM + NA/NM + NA/NM (3) -*Aureobasidium pullulans* + *Alternaria alternata* + *Exophiala xenobiotica* -*Aureobasidium pullulans* + *Aureobasidium subglaciale* + *Filobasidium wieringae* -*Trichoderma harzianum* + *Ophiostoma piceae* + *Graphilbum fragrans* Asp + NA/NM (2)* Asp + Asp + NA/NM (1)* NA/NM + Noc (1) *Lomentospora prolificans* + *Nocardia novacoligeans* Asp + NA/NM + NA/NM (1)* Asp + NA/NM + PJP (1)* Asp + Asp + Asp + NA/NM (1)*	1 1 1 2 1 1 1 1 1	***Trichosporon asahii*** (Trich) Muc + Muc + Trich (2)* Asp + Trich (1)* Muc + Trich (1)*	**4** 2 1 1
				Endemic mycoses (EM) Myco + EM (1)*	**1** 1
				Listeria monocytogenes (List) Muc + List (1)*	**1** 1

* Previously Listed

Figure 1. Chord Plot of Co-infections with Pathogens of Critical Importance



The outer circle sections represent Karius Test detections belonging to different taxonomic groups. The length of each circle section is proportional to the total number of detections of a taxon belonging to that group. The chords connecting a pair of circle sections are proportional to the number of times two taxa from those groups were observed together, weighted by the total number of taxa detected.

Conclusion. Plasma mcfDNA NGS offers a rapid, comprehensive non-invasive means of detecting CI-POCI in IC patients with one test. Although rare, co-infections with POCI can greatly increase mortality. The KT can provide important insights into pathogen-pathogen interactions in complex hosts and help optimize therapy.

Disclosures. **Matthew Smollin, PharmD**, **Karius, Inc.** (Employee) **Martin S. Lindner, PhD**, **Karius, Inc.** (Consultant) **Nicholas R. Degner, MD, MPH, MS**, **Karius Inc.** (Employee, Shareholder) **Ricardo Castillo-Galvan, MD MPH**, **Karius Inc.** (Consultant) **Jose Alexander, MD, D(ABMM), FCCM, CIC, SM, MB(ASCP), BCMAS**, **Karius** (Employee) **Ann Macintyre, DO**, **Karius, Inc.** (Employee) **Bradley Perkins, MD**, **Karius, Inc.** (Employee) **Asim A. Ahmed, MD**, **Karius, Inc.** (Employee) **Aparna Arun, MD**, **Karius** (Employee)

664. Clinical Impact of Cell-Free DNA Metagenomics in Diagnosing Infectious Diseases in Pediatrics: A Single-Center Experience

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Session: P-30. Diagnostics: Typing/sequencing

Background. Metagenomic next-generation sequencing (mNGS) of plasma cell-free DNA has significant potential to improve infectious diseases diagnostics through unbiased detection of pathogens. However, the optimal patient population or clinical condition for this testing has not been determined.

Methods. We performed a retrospective review of all orders for plasma cell-free DNA mNGS using the Karius test (Karius, Redwood City, CA) from The Children's Hospital of Philadelphia from 7/1/19-4/30/21. Chart review then determined if the test had a positive, negative, or no clinical impact.

Results. 25 mNGS tests were ordered on 24 unique patients. The majority of tests were ordered on immunocompromised patients (Table 1). Most mNGS tests were ordered after completion of routine microbiological testing (17/25, 71%). Three tests were not completed as ordered. Most completed tests (18/22, 82%) had no impact on clinical care as they confirmed the known diagnosis or were not acted upon (Figure 1). mNGS testing had a positive impact in 2 cases. For one patient with congenital heart disease presented with persistent fever and concern for endocarditis despite negative infectious workup, a negative mNGS result allowed for continued monitoring without therapy. Another patient with a lymphatics disorder had mNGS performed due to persistent clinical instability; testing was positive for *Candida parapsilosis*, allowing for early initiation of antifungal therapy. However, test results had a negative clinical impact in 2 other patients. In a patient with congenital heart disease and fever, identification of two organisms led to prolonged antibiotic therapy for endocarditis without resolution of symptoms. In a patient with leukemia, report of a dematiaceous mold led to further diagnostic testing, including a lumbar puncture, as well as treatment with antifungal therapy despite no clear diagnosis.

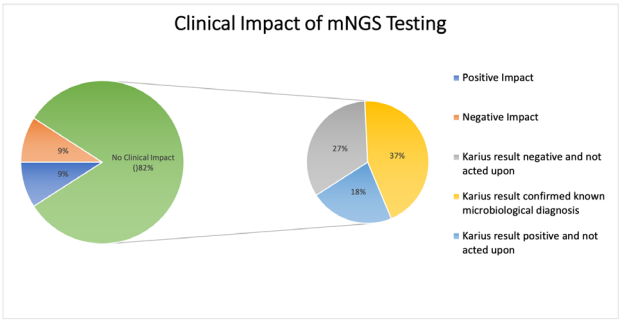

Table 1

Table 1: Clinical characteristics of patients who underwent mNGS testing and test results

Characteristic	Total (n=24)*
Age, years (median and range)	11.0 (0.2 – 28.0)
Male sex, n (%)	14 (58%)
Underlying condition	
Hematopoietic cell transplant, n (%)	5 (21%)
Active malignancy, n (%)	6 (25%)**
Primary Immunodeficiency, n (%)	3 (13%)
Rheumatologic disease, n (%)	2 (8%)
Other immunocompromised state, n (%)	3 (13%)
Congenital heart disease, n (%)	4 (17%)
None	1 (4%)
Admitted to ICU at time of testing, n (%)	12 (50%)
mNGS testing ordered after completion of routine microbiological testing, n (%)	17 (71%)
Completed Test	22 (88%)
Positive Test Results	15 (60%)
Patient ultimately died, n (%)	11 (46%)

Abbreviations: ICU, intensive care unit; mNGS, metagenomic next generation sequencing

*Twenty-four patients contributed 25 mNGS tests during the study period

**Including 2 patients who received chimeric antigen receptor T cell therapy

Conclusion. In this study, the majority of plasma cell-free mNGS tests had no impact on clinical care. mNGS testing did positively impact care in 2 patients, but did had a negative impact on care in 2 instances, leading to further testing and unnecessary treatment. Further investigation is needed to determine the ideal population or clinical condition for testing and the ideal time of sending plasma cell-free mNGS tests.

Disclosures. **All Authors**: No reported disclosures

665. Clinical and Financial Impact of Next Generation Sequencing (NGS) in addition to Conventional Microbiology Testing in our Urban Referral Health Center

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Session: P-30. Diagnostics: Typing/sequencing

Background. Clinical microbiology traditionally relies on culture methodology and serological testing, that have inherent limitations. Newer diagnostic techniques such as Next Generation Sequencing (NGS) have shown promise to improve microbial identification. In select scenarios, we send clinical specimens to reference laboratories for NGS testing in addition to current standard of care (SOC) diagnostics. We wanted to determine how this diagnostic approach has impacted patient care. We also wanted to review the financial burden through cost-benefit analysis for these 'send-out' tests.

Methods. We performed a retrospective chart review of all cases over a 3-year period in which NGS was submitted. Data, including demographics, comorbidities, antimicrobial use, and diagnosis (by SOC and NGS) were gathered. We delineated how often there was concordance or discordance between SOC and NGS. We also obtained

information on financial cost (direct and indirect) and turnaround time (TAT) for NGS results.

Results. A total of 33 clinical specimens from 25 patients were sent for NGS. The majority of specimens comprised joint tissue/fluid, organ tissue and CSF.

Concordance occurred between SOC and NGS testing in 75.8% (25/33) of samples; of those, 88% excluded infection. NGS identified a pathogen in 20% (5/25) patients in which concomitant SOC testing was negative. A subsequent change in antimicrobial management occurred in 16% (4/25) of patients. The mean TAT was 14 days and average cost per specimen was $821.52 (range: $573-$1590).

Table 1. Pathogens identified by NGS with negative traditional microbiological test results

Table 1. Organisms identified by NGS with negative SOC
Gordonia sputi
Bartonella species
Corynebacterium species
Streptococcus agalactiae

Figure 1. Distribution of specimen site (in %) sent for NGS

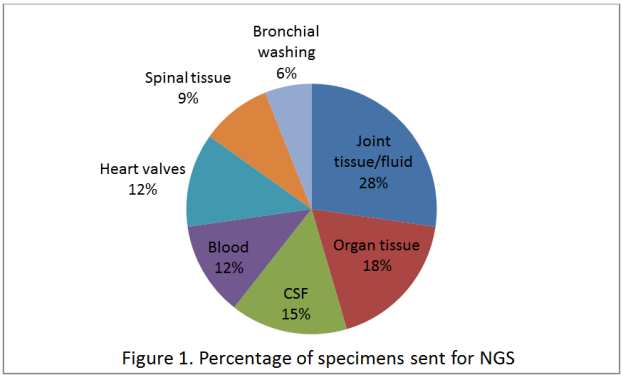

Figure 1. Percentage of specimens sent for NGS

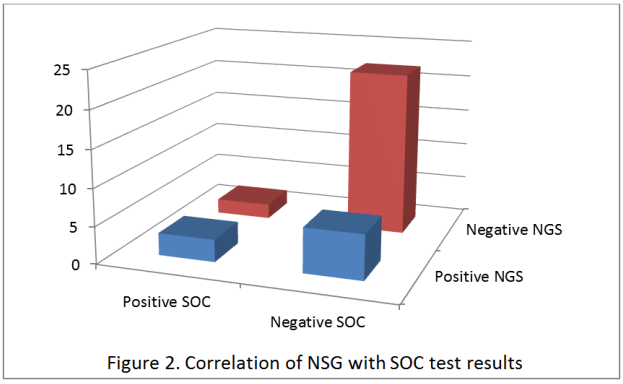

Figure 2. Correlation of NSG with SOC test results

Conclusion. NGS can provide additional diagnostic sensitivity in infectious diseases, which at our institution identified a new pathogen in 20% and a resultant treatment change in 16% of our patients. This testing may also allow physicians to reaffirm the absence of an infection diagnosis. A larger NGS testing population may reveal more significant benefits. While the attributable cost of NGS was substantial, it should be measured against the costs of administration of unnecessary antibiotics, inaccurate diagnosis, and adverse patient outcomes that may result from SOC testing alone. Considering its financial cost and extended TAT, in-house NGS testing may be warranted to facilitate a higher volume of testing.

Disclosures. **All Authors**: No reported disclosures

666. Molecular Epidemiology of Methicillin-Resistant *Staphylococcus aureus* in Chile between 1999-2018

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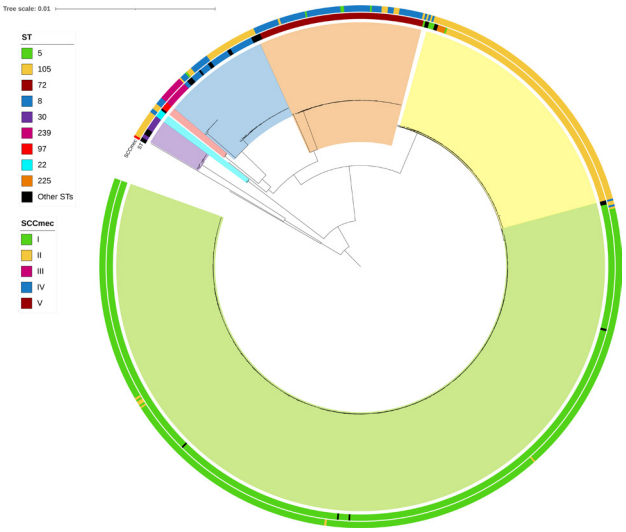
Session: P-30. Diagnostics: Typing/sequencing

Background. The global spread of methicillin-resistant *Staphylococcus aureus* (MRSA) is associated with distinct genetic lineages that predominate in specific geographical regions. Available evidence suggests the Chilean-Cordobes clone (ChC), an ST5-SCC*mec*I lineage, has largely predominated in Chilean hospitals since its first description in the late 1990's. Although the circulation of other MRSA lineages, including community-associated clones, has been well documented, the dynamics of clonal replacement over time has not been explored. Therefore, we aimed to study the molecular epidemiology and dynamics of clonal replacement using a large collection of clinical MRSA strains recovered from Chile during the last two decades.

Methods. We used whole-genome sequencing (WGS) and core-based phylogenomic analysis to identify genetic lineages and explore their relationship in 798 MRSA isolates obtained between 1999-2018 from two tertiary-care Chilean hospitals.

Results. Overall, the most frequently identified clones were the ST5-SCC*mec*I ChC (n=476, 60%), followed by ST105-SCC*mec*II (n=119, 15%), ST72-SCC*mec*IV (n=74, 9%), and ST8-SCC*mec*II (n=26, 3%). Phylogenomic reconstruction demonstrated 7 major clades: Clade I (CC30); Clade II (CC22); Clade III (CC97); Clade IV (CC8); Clade V (ST72); Clade VI (CC5/ST225 and ST105) and Clade VII (CC5/ST5-SCC*mec*I) (Fig. 1). The ChC clone remained the most frequent MRSA lineage throughout the study period (Fig. 2). However, its relative abundance decreased from >90% of isolates in 1999 to ca. 40% in 2018. This decrease began around 2005 and was associated with a progressive expansion of the ST105-SCC*mec*II and ST72-SCC*mec*IV lineages (Fig. 2). A Bayesian molecular clock analysis established the most recent common ancestor in 1964 (95% HPD interval=1961.975-1966.218) and corroborated a CC5 expansion event starting in Chile in 1999 (Fig. 3). Interestingly, our analyses revealed two branches within the ST5-SCC*mec*I lineage: one predominating in 1999-2006, and a more recent branch (related to the ST105-SCC*mec*II clone) that emerged around 2008.

Figure 1. Core genome phylogenomic reconstruction of the 798 MRSA isolates.



The seven major clades are represented by colored sections. The Clade I (purple section) was composed of isolates belonging to the CC30. Clade II (cyan section) includes four isolates of CC22. Clade III (red section) is composed of isolates of CC97. Clade IV (blue section) grouped isolates of different ST239 and ST8, belonging to the CC8. Clade V (orange section) includes isolates of ST72. Clade VI (yellow section) includes isolates of ST225 and ST105, both belonging to CC5. Clade VII (green section) is mostly composed of isolates of ST5-SCCmecI. The inner ring shows the ST of the isolates; the external ring shows the staphylococcal chromosomal cassette mec (SCCmec) type.