14TH KASH CONFERENCE THEME: HARNESSING BIOMEDICAL RESEARCH INNOVATIONS AND BIG DATA FOR HEALTH SYSTEM RESILIENCE, LOCAL MANUFACTURING AND COMMERCIALIZATION

1. PERFORMANCE AND COMPARATIVE EVALUATION OF A NOVEL DIAGNOSTIC ASSAY, NOVAPLEX™ MALARIA ASSAY KIT, AGAINST ROUTINE DIAGNOSTIC TECHNIQUES IN THE DETECTION OF DIFFERENT PLASMODIUM SPP. IN KENYA

Authors: Lewis k Mbabu (Kenya Medical Research Institute) *; Kelvin Thiongo (Kenya Medical Research Institute); Maureen Otinga (Kenya Medical Research Institute); Mary Ombati (Kenya Medical Research Institute); Lynette Wangechi (Kenya Medical Research Institute); Noah Machuki (Kenya Medical Research Institute); Francis T Kimani (KEMRI)

Background: Accurate and rapid malaria diagnosis is vital for effective treatment and control of this deadly disease. Additionally, species identification is crucial for tailoring treatment strategies toward the specific Plasmodium species responsible for the infection. Different species of Plasmodium exhibit variations in their pathogenicity and susceptibility to antimalarial drugs. Failure to correctly identify the species could result in inadequate treatment, leading to prolonged illness, parasite resistance, and potentially life-threatening complications. By accurately determining the infecting species, healthcare providers can better assess the potential disease severity and implement appropriate management strategies. This study sought to assess the performance of the Novaplex™ Malaria Assay, a novel malaria diagnostic kit, against established diagnostic methods, including microscopy, rapid diagnostic tests (RDTs), and polymerase chain reaction (PCR), in Plasmodium detection.

Methods: Blood samples were collected from 142 suspected malaria cases in Matayos, Kenya. The samples were tested using microscopy, RDTs, Novaplex™ Malaria Assay, and qPCR to determine positivity and identify the Plasmodium species. Various diagnostic parameters, such as sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), accuracy, and agreement (Cohen's kappa), were calculated to evaluate the assay's diagnostic performance in comparison to the other techniques.

Results: Novaplex™ Malaria Assay exhibited superior sensitivity, accuracy, and NPV when compared to microscopy and RDTs. Furthermore, the assay showed diagnostic agreement with qPCR, which was considered the "gold standard" for this analysis. In terms of species identification, the Novaplex™ assay demonstrated performance on par with qPCR. Specifically, the assay exhibited a sensitivity of 95.5% and a specificity of 87.5%, while microscopy and RDTs had sensitivities of 63.7% and 61.5%, respectively. The PPV and NPV for the assay were 99.2% and 53.9%, in contrast to microscopy and RDTs, which had low NPVs of 12.5% and 11.9%, respectively. The overall accuracy of the Novaplex™ assay was 95.1%, with a substantial agreement with qPCR (k=0.642). In contrast, microscopy and RDTs had lower accuracy levels (65.5% and 63.4%, respectively) and slight agreement with qPCR (k=0.148 and k=0.136, respectively).

Conclusion: Novaplex™ Malaria Assay outperformed traditional methods such as microscopy and RDTs while showing comparable performance to qPCR in identifying and speciating Plasmodium spp. responsible for malaria infections. Its high sensitivity and specificity make it a promising diagnostic tool for malaria.

2. ANTI-PROLIFERATION EFFECTS OF RHAMNUS PRINOIDES AND GREWIA VILLOSA EXTRACTS BASED ON IN VITRO, NETWORK PHARMACOLOGY AND MOLECULAR DOCKING APPROACHES AGAINST CERVICAL CANCER.

Authors: Sally W Kamau (KEMRI)*; Sospeter N Njeru (KEMRI); Mathew Piero (Kenyatta University); Peter G Mwitari (KEMRI); Mercy Jepkorir (KEMRI)

Background: Cancer is a leading cause of mortality globally. The burden is heaviest in LMICs. It is second as a leading cause of mortality according to the National Cancer Institute of Kenya after infectious diseases and cardiovascular diseases. Cervical cancer is the third most diagnosed cancer however it is the leading cause of mortality. Chemotherapy is the most common treatment, however, the cost of treatment coupled with severe side effects are often associated therein. Medicinal plants offer an alternative that is cost-effective, locally available and fewer side effects. Medicinal plants also serve as a reservoir for the development of more efficacious drugs for the treatment.

Method: Rhamnus prinoides and Grewia villosa plants were collected, air-dried and ground into a powder. Cold extraction technique was used for the extraction followed by solvent partitioning to obtain four extracts based on the solvent (crude (DCM: MeOH), Ethyl acetate, Hexane and Water). The MTT ((3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay was used to test for the anti-proliferative activity of the extracts against cervical cancer cells (HeLa) and normal cells (Vero-ccl 81) and the selectivity index determined. Qualitative phytochemical screening was conducted according to standard methods as well as GC/MS. The compounds obtained were run through various databases to determine their putative pharmacokinetic properties (ADMET values) and then based on their results, some were selected for network pharmacology and molecular docking studies and RT-qPCR used to validate them.

Results: Following qualitative phytochemical screening, Rhamnus prinoides extracts had glycosides, saponins, tannins and phenols in abundance while the Grewia villosa extracts had alkaloids, phenols and tannins in abundance. The IC50 (Inhibitory concentration that kills 50% of cancerous cells) values for the extracts of Rhamnus prinoides were198 µg/ml, 155 µg/ml and 78 µg/ml (ethyl acetate extract) with corresponding CC50 (Cytotoxic concentration that kills 50% of non-cancerous cells) values of 519 µg/ml, 568 µg/ml and 177 µg/ ml respectively showing their strong selectivity. The IC50 values for Grewia villosa were 380 µg/ml, 205 µg/ml and 107 µg/ml for the crude, hexane and ethyl acetate extracts respectively with 735 µg/ml, 428 µg/ml and 226 µg/ml CC50 values respectively. The top 5 genes identified through network pharmacology analysis were; for Rhamnus prinoides (TNF, STAT3, EGFR, IL1B and NFKB1) and for Grewia villosa (AKT1, EGFR, STAT3, NFKB1, and PTGS2). Molecular docking analysis was carried out and all the genes had a binding affinity of ≤ 0.0 however only the ones with binding affinities ≤ -7.0 were selected. Scratch assay also showed that the extracts limit migration. The activity of the genes was then validated in vitro through RT-qPCR.

Conclusion: The results confirm the potential of traditional plants in drug development of the treatment of cancer.

3. IN VITRO ANTIPROLIFERATIVE ACTIVITY OF ASPILIA PLURISETA AGAINST PROSTATE CANCER

Authors: Innocent Okpako (Pan African University Institute of Basic Sciences, Technology and Innovation) *; Florence Ng'ong'a (Jomo Kenyatta University of Agriculture and Technology); Cleophas Kyama (Jomo Kenyatta University of Agriculture and Technology); Sospeter N Njeru (KEMRI)

Background: Prostate cancer is the second most prevalent cancer among men after the age of 65. Annual incidences are estimated to be 1.41 million and 375,000 deaths worldwide. Current treatments are associated with life-threatening side effects, hence the need to search for effective and safe treatment alternatives. Aspilia pluriseta has been demonstrated to have anticancer activity in lung and liver cancer cell lines. This study investigated the antiproliferative effects of A. pluriseta on prostate cancer cells.

Methods: A. pluriseta root crude extract was prepared using dichloromethane/methanol (1:1 v/v) and partitioned into hexane, ethyl acetate, and water fractions. The MTT assay was used to screen the fractions for antiproliferative activity on cancerous DU-145 at a single concentration of 200 µg/ml. The active fractions were further tested at 6.25–200 µg/ml on the cancerous cells and non-cancerous Vero E6 cells. Qualitative phytochemical and gas chromatography-mass spectrometry (GC-MS) analyses were done to identify the active chemical compounds in the active fractions. Network pharmacology was then explored to predict the putative molecular targets and mechanisms of action of drug-like compounds. Molecular docking and real-time qPCR were used to validate the predictions.

Results: The active fractions were crude dichloromethane/methanol, hexane, and ethyl acetate fractions. They inhibited the proliferation of DU-145 cells with IC50 values of 16.94, 20.06, and 24.14 µg/ml, respectively. Selectivity indices were 6.04 (crude), 3.62 (hexane), and 6.68 (ethyl acetate). Identified phytochemicals included phenols, terpenoids, flavonoids, tannins, sterols, and saponins. Seventy-nine (79) compounds were identified by GC-MS, and seven (7) of the compounds met the set ideal drug candidate parameters; their top molecular targets included MAPK3, MAPK1, IL6, TP53, ESR1, PTGS2, MMP9, MDM2, AR, and MAP2K1, while deregulation of PI3K/Akt, MAPK, and p53 signalling pathways was indicated as the most probable mechanisms of action. The main compounds were found to be 1-heneicosanol, lanosterol, andrographolide, and retinoic acid. Lanosterol had the strongest binding activities with MAPK21 (-9.7 kcal/ mol), ESR1 (-8.9 kcal/mol), and MAPK1 (-8.8 kcal/mol). The mRNA expression of AR was downregulated and p53 was upregulated in DU-145 cells treated with A. pluriseta but not in control cells that were exposed to 0.2% DMSO. Similarly, both CDK1 and BCL-2 were downregulated, while caspase-3 was upregulated.

Conclusions: A. pluriseta inhibited the growth of DU-145 without causing cellular toxicity and has promising potential to be developed as an anti-prostate cancer agent. However, further in vitro and in vivo experiments are recommended.

4. PHYTOCOMPOUNDS OF CAJANUS CAJAN AS POTENTIAL ANTICANCER AGENTS AGAINST BREAST CANCER; AN ANALYSIS THROUGH NETWORK PHARMACOLOGY AND MOLECULAR DOCKING.

Authors: Douglas K Njuguna (KEMRI)*; Peter Mwitari (KEMRI)

Introduction: Breast cancer is a leading cause of deaths among women in developing countries, mostly due to unaffordability of chemotherapeutics. As a result, many cancer patients seek complementary and alternative medicines (CAM) which are deemed cheaper and safer. It is therefore critical to enhance research to elucidate the anticancer potential of CAM. Cajanus cajan is a food crop rich in phytocompounds which have various pharmacological effects; cajanol in particular was shown to have activity against MCF7 breast cancer cells, however, the anticancer activity of the other phytocompounds and their mechanisms of action have not been fully elucidated.

Methods: Active compounds of Cajanus cajan were retrieved from IMPPAT 2.0 database and from literature and were subjected to filtering conditions of Lipinski Rule, GSK 4/400, Pfizer 3/75 and Drug Likeness score and their target genes predicted using Swiss Target Prediction. GEO, DisGeNet, GeneCards and OMIM databases were used to retrieve breast cancer target genes. The target genes were imported to Venny to obtain drug-disease common target genes. PPI network was constructed using STRING and visualized in Cytoscape. CytoHubba was used to predict the Hub genes. Gene Ontology (GO) enrichment analysis and KEGG Pathway analysis was conducted using DAVID Bioinformatics database. Top 10 enriched Biological Processes (BP), Cellular Components (CC) and Molecular Functions (MF) and top 20

KEGG Pathways were visualized in Bioinformatics.com. Molecular docking was performed using Vina plug-in in PyRx and visualization done in Biovia Discovery Studio.

Results: A total of 76 compounds of Cajanus cajan were retrieved and 10 compounds were retrieved after subjection to filtering conditions, these were 2'-Hydroxygenistein, Cajanol, Cajanin, Cajaquinone, Apigenin, Luteolin, Genistein, Naringenine, Quercetin and Isorhamnetin. A total of 257 target genes of the active compounds were obtained, 2510 breast cancer target genes were retrieved from GEO, 115 from DisGeNet, 200 from OMIM and 884 from GeneCards. A total of 113 common target genes were retrieved as the common target genes between active compounds and breast cancer. From the PPI network, a total of 10 Hub genes were identified (SRC, PIK3CA, PIK3R1, PIK3CD, PIK3CB, HSP90AA1, ESR1, AKT1, EGFR and PTK2) and were considered as the potential targets of the 10 active compounds of Cajanus cajan in the treatment of breast cancer. GO enrichment analysis involved 91 BP, 19 CC and 30 MF. KEGG pathway involved 110 pathways. The 10 active compound demonstrated good biding properties at the 10 core targets (binding energy less than -5.0.

Conclusion: The 10 active compounds of Cajanus cajan exert anticancer effects by acting on signaling pathways such as VEGF, ErbB, Estrogen and Prolactin through key targets such as SRC, PIK3CA and HSP90AA1 thus providing predictive indicators for further research to verify their mechanisms of action.

5. MANAGEMENT OUTCOMES OF BURNS IN PEDIATRIC PATIENTS <13YEARS AT THIKA LEVEL V HOSPITAL, KIAMBU COUNTY, KENYA.

Methods: A retrospective cross-sectional study design was conducted, analyzing 41 patient files obtained hospital was 3%. Burns are more common within the age bracket of 1-3 years 22(56%) with male 23(59%) being affected. Scald burns accounted for 38(97%). Fluid therapy 23(59%) and silver sulfadiazine 33(84%) were the most commonly used management options. Other management options included pain management, nutritional therapy, grafting among others. Mild degree of burns accounted for 31 (79.5%), while 2nd degree superficial burns were mostly reported 34 (87.2%). The average duration of hospital stay was 15 days. Fluid therapy significantly affected the duration of hospitalization. There was a positive correlation between depth of burns and duration of hospitalization (Spearman's rho ($\rho = 0.245$, $p = 0.133$). Conclusion: Burns contribute to 5% of disability in pediatric patients, with a prevalence of 3% at Thika Level V Hospital. It primarily affects males and children aged 1-3 years. Dermazine is the main management option, and fluid therapy plays a significant role in hospitalization duration, typically less than 10 days. This suggests that prevention at the household level and effective burn management using pediatric protocols are strongly recommended.

6. DESIGN AND OPTIMIZATION OF A MALARIA PF/PAN ANTIGEN DETECTION KIT IN KEMRI, KENYA; ADVANCING HEALTHCARE THROUGH LOCAL MANUFACTURING OF MEDICAL DEVICES

Authors: Abdiaziz A Gosar (KEMRI)*; Matoke Damaris (KEMRI); Lameck Motari Nyabuti (Kenya Medical Research Institute); Kevin Thiongo (KEMRI); Maureen Otinga (KEMRI); Francis T Kimani (KEMRI); James H. Kimotho (Kenya Medical Research Institute)

Background: Malaria is a global health concern with 219 million cases and 409,000 deaths reported in 2019 by WHO. Most cases occurred in Sub-Saharan Africa, specifically Kenya with 3.5 million cases and 10,500 deaths. Prompt and accurate malaria diagnosis is crucial for control and elimination. While microscopy is the recommended diagnostic method, it has limitations in low-resource settings. Rapid Diagnostic Tests (RDTs) have been developed as an alternative to microscopy. In Kenya, the demand for malaria RDTs is

estimated at 4 million tests per year. This study aimed to develop a locally produced Malaria Pf/Pan rapid diagnostic kit using pHRP II and pLDH antigens. 14th 136 kash Conference

Methodology: Anti-pHRP II and anti-pLDH monoclonal antibodies were conjugated to 20nM colloidal Gold suspension, dried using a lyophilizer, and coated onto a nitrocellulose membrane with a spraying machine. The conjugates were then blocked and dried. The kit's performance was evaluated using positive and negative malaria samples, with 27 positive and 22 negative samples tested.

Results: Out of 27 positive malaria samples, all tested positive overall, 89% for Pf, and 100% for Pan. All 22 negative samples tested negative. The kit demonstrated a sensitivity of 100% overall, 89% for Pf, and 100% for Pan. The specificity was 100%.

Discussions: The study successfully developed a prototype of a Pf/Pan malaria detection kit with 100% sensitivity and specificity. Further optimization is required to determine the optimal protein dilution for cost-effective production and to consider the use of proteins circulating in Kenya, accounting for mutations in HRP II/III proteins.

Conclusion: The study successfully produced a prototype of a malaria Pf/Pan antigen rapid detection kit, requiring further evaluation. Keywords: Malaria, Antigen, Rapid Kits, Pf, Pan

7. ISOLATION AND CHARACTERIZATION OF ENVIRONMENTAL LYTIC BACTERIOPHAGES AGAINST ENDEMIC MULTIDRUG-RESISTANT ENTEROCOCCUS FAECALIS AND ENTEROCOCCUS FAECIUM IN KENYA

Authors: Oumarou Soro (Pan African University for Basic Sciences, Technology, and Innovation (PAUSTI) *; Lillian Musila (KEMRI); Andrew Nyerere (JKUAT)

Background: Enterococcus faecalis and Enterococcus faecium, Gram-positive cocci, are a growing cause of nosocomial and antibioticresistant infections. Treating drug-resistant E. faecalis and E. faecium requires novel approaches. This study aimed to isolate and characterize lytic bacteriophages (phages) from wastewater in Kenya, evaluate their antibacterial activity against planktonic strains and biofilm formers of clinical multidrug-resistant (MDR) E. faecalis and E. faecium isolates, and establish their genomic characteristics.

Methods: In total, 26 MDR E. faecalis and 11 MDR E. faecium were used to screen for phages from water sampled from Ruai sewage plant, JKUAT dam, Kibera, and Kahawa sewage using spot assays. Phages were purified using plaque assays and phenotypically characterized by host range tests, temperature, and pH stability assays. The crystal violet biomass assay was used to study the phage's effect on biofilm formation and disruption. The genomes of 17 phages were sequenced on the Illumina MiSeq platform. The quality of the raw reads was assessed using fastqc v0.12.1, trimmed with fastp v0.23.4, and assembled with shovill v1.1.0. Genome annotation was performed using pharokka v1.5.1, and lysogeny genes were predicted using the PhageLead platform.

Results: Five E. faecium and 17 E. faecalis phages were isolated. The host range test showed that 10/17 E. faecalis phages had lytic activity against at least 50% of the E. faecalis strains, but the E. faecium phages only lysed the host bacteria. All phages remained infective against their host bacteria at temperatures between -80ºC and 50ºC and pH between 5 and 11. The biofilm formation assay showed that 24/26 E. faecalis and 1/11 E. faecium isolates could form biofilm. All E. faecalis phages inhibit and disrupt the biofilm of their host. Genomic analyses showed that all the phages belong to group I of Baltimore's classification and varied in length from 17,979 to 147,374 bp, with G+C contents ranging from 33.14% to 40.05%. The genomes contained 28 to 250 coding sequences. Phages were classified into the families

Rountreeviridae (2/17), Herelleviridae (2/17), and unclassified (13/17). They lacked AMR, virulence, and lysogeny genes, suggesting they are strictly virulent and have therapeutic potential.

Conclusion: This study demonstrates that natural phages against Enterococcus sp. exist in diverse environments in Kenya and that biofilms are associated predominantly with E. faecalis infections. These phages are promising candidates for phage therapy and biofilm control and could be used singly or in cocktails to target endemic E. faecalis and E. faecium infections.

8. COMPARISON BETWEEN SANGER SEQUENCING AND OXFORD NANOPORE SEQUENCING OF POLIOVIRUSES: THE KEMRI POLIO LABORATORY EXPERIENCE.

Authors: Mercy A. Onyango (Kenya Medical Research Institute)*; Joanne Hassan (KEMRI); Agnes Chekurui (KEMRI); Rosemary Nzunza (KEMRI); Evans Komen (KEMRI); Stephen Ochieng Ombija (Kenya Medical Research Institute); Robert Mainga (KEMRI); Janet Ngugi (KEMRI); Samira Ali Katembe (KEMRI); Jenniffer Lewett (KEMRI); Diana Wanjiru (KEMRI); Paul Muchai (KEMRI); Shadrack Mr. Barmasai (Kenya Medical Research Institute); Peter Maritim (KEMRI); James Nyangao (KEMRI); Benlick Mwangi (KEMRI); Collins K Cheruiyot (KEMRI); Maureen Njihia (KEMRI); samoell A Khamadi (KEMRI); Peter Borus (World Health Organization)

Background: Poliovirus (PV) isolates are taken through genetic sequencing to confirm detection, source, monitor geographic trends in transmission and establish vaccination strategies. The increasing incidence of circulating vaccine-derived poliovirus (cVDPV) infections, has highlighted necessity of genetic sequencing and analysis at country level to improve detection and response timeliness. Kenya Medical Research Institute EPI laboratory is responsible for region-wide diagnosis of poliovirus by virus isolation in cell culture and intratypic differentiation (ITD) through Polymerase Chain Reaction (PCR) techniques. Sanger Sequencing of the PV VP1 capsid region is carried out at a separate laboratory in Atlanta to confirm poliovirus detection and distinguish cVDPV from vaccine strains. We undertook a comparative study in the KEMRI lab to compare sequencing similarity between Oxford Nanopore sequencing of Polioviruses from stool samples and compared it to the gold standard Sanger sequencing results from our reference lab in Atlanta.

Methods: 48 stool samples, between July-November 2023, which were positive on L20B and RD cell cultures, were typed by ITD qPCR to identify their serotype in accordance to the WHO standard algorithm. Of these, 19 samples containing PV type 2 had the VP1 region sequenced using both platforms. Where consensus cVDPV2 and Sabin 2 VP1 sequences were available from both nanopore and Sanger sequencing of culture isolate for the same sample, the similarity of the sequences generated was determined.

Results: The percentage of similar nucleotide results for PV VP1 sequencing from Nanopore and Sanger sequencing for the 19 samples with results for both methods was 18/19 (94.73%). The only sample with the differing results had 51 reads for PV type 2, which was just above the cut off (50) for a consensus on nanopore, which explains why it was not scored as a PV 2 virus. However, the second specimen collected from the same patient that underwent the same culture process, had identical results on both sanger and nanopore. Additionally, a comparison between virus extraction starting material as stool suspension (extract) versus cell culture supernatant (isolate) was tested on two samples. One sample yielded 100% similar nucleotide sequence results in both isolate and extract, while the second yielded a 2-nucleotide difference between the extract and isolate.

Conclusion: After sequencing, 18/19 isolates yielded 100% similar VP1 nucleotide sequences on both methods. Nanopore sequencing therefore gives highly similar results as those obtained by the gold standard, Sanger. Another advantage of Nanopore sequencing is it allows the identification of multiple polio

serotypes from a single sample, which is problematic for the sanger method. Furthermore, at analysis, it enables rapid identification of contamination during the assay. Overall, it is fast and easy to conduct at the lab and therefore improves the timeliness of results generation.

9. COMPARATIVE EVALUATION OF THE AUTOMATED VITEK 2 AND MICROBROTH DILUTION METHODS ON COLISTIN ANTIBIOTIC SUSCEPTIBILITY TEST RESULTS

Authors: Allan Barasa Wataka (USAMRD-A KEMRI) *; Michelle Atieno Omondi (USAMRD-A/KEMRI); Erick Odoyo (USAMRD-A KEMRI); Lillian Musila (USAMRD-A KEMRI)

Background: Colistin is a polymyxin polycationic antibiotic used as a last-resort antibiotic for treating infections caused by multidrug resistant (MDR) gram-negative bacteria, including Pseudomonas aeruginosa and Acinetobacter baumannii. Given the global rise in colistin resistance, monitoring its antibiotic resistance is important to evaluate its clinical utility. In Kenya, colistin is not routinely tested but is included in automated platforms such as the VITEK 2 and could be reported for clinical use. However, the Clinical and Laboratory Standards Institute (CLSI) does not recommend VITEK 2 for colistin testing and has recently changed the interpretation breakpoints significantly. Misreporting results may cause treatment failures or withholding of an effective drug, leading to unnecessary mortality for patients with MDR infections. In this study, we compared colistin susceptibility using the CLSI-recommended microbroth dilution method 14th 138 kash Conference (MBD) and the VITEK 2 to determine the potential impact of incorrect testing on therapeutic decisions.

Methods: We retested 57 gram-negative clinical bacterial isolates that were colistin-resistant on the VITEK 2 GN AST Card. The isolates were 44 Pseudomonas aeruginosa, 5 Acinetobacter baumannii, 4 Enterobacter spp, 1 Myroides sp., 1 Aeromonas hydrophila/caviae, 1 Klebsiella pneumoniae and 1 Sphingomonas paucimobilis. We included E. coli ATCC strain 25922 as the negative control and Proteus mirabilis (intrinsic resistance) as the positive control. The isolates were tested in a microbroth dilution assay using Cation-Adjusted Mueller Hinton Broth (CAMHB). Concentrations of 0 µg/ml, 1 µg/ml, 2 µg/ml and 4 µg/ml were achieved by adding appropriate numbers of 10 µg colistin disks to the media in tubes to which 50µl of the bacterial suspension was added and incubated for 16-20hrs at 35°C. The minimum inhibitory concentrations were obtained, interpreted per the CLSI guidelines 2022, and compared with the VITEK 2 colistinsusceptibility results.

Results:On the VITEK 2, all 57 (100%) isolates were colistin-resistant. By the microbroth dilution assay, 44/57 (77.2%) isolates were classified as intermediate, including 38 P. aeruginosa, 4 A. baumannii isolates, 1 Myroides sp., and 1 Sphingomonas paucimobilis. In comparison, 13/57 (22.8%) isolates were consistently classified as colistin-resistant, including 6 P. aeruginosa isolates, 1 A. baumannii, 4 Enterobacter spp, 1 Aeromonas hydrophila/caviae, and 1 Klebsiella pneumoniae ssp pneumoniae.

Conclusion:The study confirmed that colistin results on the VITEK 2 can be misleading as resistance was over-reported for priority MDR pathogens P. aeruginosa and A. baumannii, which could lead to underuse of this important last-line drug, thus contributing to unnecessary mortality. We recommend the MBD assay should be part of routine microbiology tests in hospitals with high MDR levels despite it being tedious and time-consuming, as colistin is only reliably tested by this method.

10. DEVELOPMENT OF A SHIGELLA MULTIVALENT BIOCONJUGATE VACCINE: A PHASE I/II RANDOMIZED, CONTROLLED AND AGE DESCENDING STUDY INCLUDING DOSE FINDING IN KENYAN INFANTS

Authors: Jane Adetifa 1, Linet Cherono 2, Chinaza Ezirim 3, Cristina Alaimo 3, Patricia Martin 3, Josphat Kosgei 2, Mainga Hamaluba 1. 1 KEMRI-CGMRC, Kilifi, Kenya, 2 KEMRI-USAMRD-K, Kericho, Kenya, 3 LimmaTech Biologics AG, Schlieren, Switzerland.

Background: Shigella is among the most common causes of severe diarrhea and dysentery worldwide, especially among young children from lower resourced countries and travelers. Although several oral Shigella vaccines have been clinically evaluated, risk of reactogenicity and potential reversion back to a pathogenic phenotype have proven challenging. Thus, a new type of vaccine to prevent shigellosis is key. The O-antigen serotype-specific immune response detected in association with convalescence from shigellosis has encouraged development of a new generation of glycoconjugates as an alternative vaccine strategy against Shigella. A Shigella flexneri 2a bioconjugate has shown, in a controlled human infection model, protection from the most severe form of the disease after challenge with S. flexneri 2a strain 2457T. In addition, immune responses post vaccination were associated with a lower disease severity score.

Methods: The potential of bioconjugation to develop a high fidelity and cost-effective multivalent Shigella vaccine targeting the most common serotypes contributing to global morbidity and mortality has been exploited. The tetravalent Shigella4V bioconjugate, including O-antigens from S. flexneri 2a, 3a, 6 and sonnei, has been evaluated for safety and immunogenicity in the target population of 9 months old infants. The trial was conducted in Kenya starting with a step 1, age descending and dose-escalating cohort, followed by a step 2 dose finding cohort where the target population received a 3dose schedule of the bioconjugate delivered with and without alum adjuvant.

Results: The last patient last visit occurred in Q4 2022 and immunogenicity and safety data (from the target population) from the interim analysis (IA) was made available in Q3 2022, the remaining data will be available in Q4 2023.

Conclusion: The IA data has shown good immunogenicity 1-month post 2nd vaccination across all treatment groups and serotypes along with well distributed and mostly mild safety events. Following the positive IA results, we have progressed towards the next steps

11. A TRANSCRIPTOME ANALYSIS OF BREAST TUMORS FROM KENYAN WOMEN

Authors: Francis W. Makokha (Mount Kenya University) *

Background: Breast cancer genomic studies in indigenous African populations are limited, despite recording the highest mortality rates worldwide. Evaluating gene signatures and mutational profiles could help to understand the biological pathways that might be amenable for the treatment of more aggressive breast cancers. We aimed to assess the transcriptomes of tumor and adjacent non-tumor tissue pairs from Kenyan women diagnosed with breast cancer to identify altered genomic pathways.

Methods: We performed RNA sequencing on 22 pairs of fresh frozen breast tumor and adjacent normal breast tissues from Kenyan women to investigate their gene expression profiles. Differentially expressed genes (DEGs) were identified at a cut-off using an adjusted $P < 0.05$, and an absolute fold change ≥ 2. In a gene set enrichment analysis (GSEA), Hallmark and Kyoto Encyclopedia of Genes and Genomes (KEGG)-defined gene sets (n=50 and 186, respectively) were selected within the GSEA molecular signature database, and those with a False Discovery Rate (FDR) < 0.25 were included in our data analysis.

Results: RNA sequencing identified 884 DEGs, including 348 upregulated and 536 downregulated protein-coding transcripts. Generally, the most upregulated genes in Kenyan tumors are associated with cell cycle control. Hallmark-defined E2F targets and the G2M checkpoint were identified as the most significantly enriched gene sets using GSEA. The top five upregulated genes in tumors were H2BC5, H2BC21, H3C4,

H2BC18, and PAQR4, while the top five downregulated genes were PLAC9, SPTBN1, SORBS1, MTURN, and CLDN5. PLK1, TOP2A, NUSAP1, and CDC20 were the most upregulated genes.

Conclusion: Our findings suggest that gene sets involved in E2F signaling and G2M checkpoint pathways are significantly enriched in the breast tumors of Kenyan patients. This observation will require further validation in a larger study to assess if these pathways constitute vulnerabilities that can be targeted as biomarkers and therapy for breast cancer

12. MONITORING THE BATTLEGROUND: EXPLORING ANTIMICROBIAL RESISTANCE, ANTIBIOFILM TRENDS, AND VIRULENCE FACTORS IN WOUND BACTERIAL ISOLATES

Authors: Silas O Awuor (Jaramogi Oginga Odinga teaching & Referral Hospital) *

Background: There is an alarming increase in antibiotic resistance, especially in common bacterial infections that could be attributed to extensive and indiscriminate use of antibiotics. Continuous strategic monitoring of antibiotic use and AMR trends becomes imperative. Chronic wound infection remains a public concern in this era of AMR. This motivates a thorough evaluation of wound isolates for relevant bacteria and assessing their drug susceptibility patterns, which we report herein. The study aimed to decipher antibacterial resistance by examining samples collected from patients with chronic wounds seeking medication at Jaramogi Oginga Odinga Teaching and Referral Hospital (JOOTRH).

Methodology: This was cross-sectional study among the patients with chronic wounds seeking medication at Jaramogi Oginga Odinga Teaching and Referral Hospital (JOOTRH). Standard microbiological methods were employed to identify and characterize the bacterial pathogens.

Results: Analysis of the wound isolates revealed a significant presence of microbial growth, with a higher prevalence of 59% isolates in male patients. Staphylococcus aureus 20.7% emerged as the most predominant pathogen, followed by Klebsiella spp.14.8%, P. aeruginosa spp. 14.8%, and E. coli 4.4% in the wound samples. Notably, Cotrimoxazole exhibited the highest antibacterial resistance 48.1%, followed by Clindamycin 25.9% and Erythromycin 25.9%, affecting both Gram-positive and Gram-negative bacteria. Furthermore, among the isolates, 75% were capable of producing haemolysin and protease, while 50% produced lipase and phospholipase, factors that enhance virulence and survival.

Conclusion and recommendation: These findings provide crucial insights into antimicrobial resistance in chronic wounds among patients attending JOOTRH, thus the importance of creating awareness and emphasis on responsible antibacterial use in wound management and shedding light on the need to developing more potent antibiotics to treat chronic wounds effectively

13. GENOMIC CHARACTERIZATION OF LYTIC BACTERIOPHAGES ISOLATED IN KENYA IDENTIFIES CANDIDATE PHAGES FOR TREATMENT OF PSEUDOMONAS AERUGINOSA INFECTIONS

Authors: Collins K Kigen (KEMRI/USAMRD-A) *; Martin MG Omondi (walter reed); James Wachira (KEMRI/USAMRD-A); Vanessa Natasha (KEMRI/USAMRD-A); Erick Odoyo (KEMRI/USAMRD-A); Mikeljon Nikolich (Walter Reed Army Institute of Research); Lillian Musila (KEMRI/USAMRD-A)

Background: P. aeruginosa is a WHO priority pathogen due to rising antibiotic resistance and presence of various virulence factors which pose treatment challenges for nosocomial infections it causes. The global spread of multidrug-resistant, extensively drug-resistant, and pan-resistant infections has limited/exhausted treatment options with conventional antibiotics, urging the development of alternative treatments. Bacteriophages specifically infect and kill bacteria and possess therapeutic potential. Ideal therapeutic

phages should have broad host ranges, lytic lifecycles, and lack toxin, AMR and virulence genes. Genomic characterization of phages is therefore essential to ensure clinical safety, in addition to enhancing a better understanding of phage biology, diversity, and host specificity.

Method: Thirty-five plaque-purified bacteriophages, isolated from wastewater samples in Kenya and exhibiting lytic activity against clinical P. aeruginosa isolates by spot test, were evaluated through whole-genome sequencing. The phages underwent amplification, filtration (0.22 µm) and DNAse treatment prior to phage DNA extraction, library preparation, and sequencing on a Nanopore platform. Raw reads were assembled using Flye and the genomes annotated using Pharokka to determine genome length, GC%, taxonomy, AMR and virulence genes, CRISPR sites, and tRNAs. Lysogeny markers was determined using PhageLeads. 14th 216 kash Conference

Results: The 35 plaque-purified phage samples from isolation and enrichment, harbored 1 to 5 distinct complete genomes each, indicating the presence of both pure, single phages and morphologically indistinguishable composite phages. Genome lengths varied from small (25kb) to large jumbo phages (>277 kb). Eight distinct taxonomic groups were identified: Phikmvvirus, Pifdecavirus, Pakpunavirus, Kochitakasuvirus, Phikzvirus, Septimatrevirus, Bruynoghevirus, and an unclassified category. Single-genome phage (89 – 93 kb) in the Papkunavirus genus, demonstrate lysis and replication capabilities independent of other phages, while the jumbo phages, all from Phikvirus genus, had a partner Papkinavirus phage suggesting a dependence for functionality. GC composition correlated with genome lengths: jumbo phages (277 – 281 kb) having the lowest GC% of 36 – 37 %, the 89-93 kb phages with 49%, while the smaller size phages (25 – 64 kb) had higher GC% of 53 – 62%. Notably, the phages with lower GC% than their P. aeruginosa host GC% of ~ 65%, carried tRNAs, with the 89 – 93kb phages having 12 -17, and jumbo phages 6 – 7 acting as codon compensation for less frequently used host codons. All the phages lacked lysogeny markers, CRISPRs, AMR, virulence, and toxin genes, posing no adverse risks to humans.

Conclusion: Genomic characterization of phages from Kenya revealed 7 distinct and 1 novel taxa, and potentially symbiotic phages with diverse genomic features. Importantly, all the phages lacked undesirable genomic features, making them good candidates for phage therapy

14. HOST RANGE DETERMINATION OF LYTIC PSEUDOMONAS AERUGINOSA PHAGES ISOLATED FROM KENYAN WASTEWATER IDENTIFIES SIX PHAGES WITH BROAD ACTIVITY ACROSS ENDEMIC STRAIN TYPES.

Autors: Martin MG Omondi (walter reed) *; Ivy J. Mutai (Institute of Primate Research/USAMRD-A); Erick Odoyo (Kenya Medical Research Institute/United States Army Medical Research Directorate-Africa, Nairobi, Kenya); Mikeljon Nikolich (Walter Reed Army Institute of Research, Silver Spring, Maryland, USA); Lillian Musila (Kenya Medical Research Institute/United States Army Medical Research Directorate-Africa, Nairobi, Kenya)

Background: Pseudomonas aeruginosa (PA) is a significant hospital-acquired infection (HAI) pathogen increasingly resistant to multiple antibiotic classes. Bacteriophages (phages), viruses that infect bacteria, have been proposed as a potential therapeutic option to treat multidrug-resistant (MDR) bacteria, including PA. A major challenge to phage therapy is the narrow spectrum of many individual phages, which have limited strain specificity. This study assessed the host range of PA phages obtained from Kenyan wastewater against multiple PA clinical isolates that are endemic in Kenya to determine if they have broad therapeutic value.

Methods: The phage hunting targeted six non-endemic PA strains (PAO1, MRSN 3705, MRSN 4841, MRSN 9718, and ATCC 10145) and produced 25 phages. To determine their lytic activity against endemic

strains, a host range assay using the direct spot test was conducted on 51 clinical PA isolates belonging to 51 distinct multilocus sequence strain types representative of the strains causing infections in different Kenyan counties: Nairobi (15), Kisumu (19), Kericho (3), Kisii (9), and Kilifi (5). The lytic activity of phages with the broadest host range by spot test was quantified by the efficiency of plaquing (EOP) assay. Phages with EOP values of >1 compared to their screening host were considered good candidates.

Results: From the spot tests, Ø8 was the least effective as it could not lyse any of the endemic strains. The most susceptible bacterial strains were ST 850 (Nairobi), 3672 (Nairobi), 3671(Malindi), 267 (Kisii), and 3666 (Nairobi). Strain types 654,455, 285, 3675, 233, 2143, 2037, 17, 2483, 2483, 41, 2025, and 871 were not susceptible to any of the 25 phages. The 15/25 phages with the broadest host range by spot test were selected for EOP. The phages were able to lyse 76.4 % of the endemic isolates efficiently. Six phages showed the broadest host range among the isolates tested: Ø19 (47.06%), Ø20 (43.14%), Ø21 (35.29%), Ø23 (45.10%), Ø24 (49.02%), Ø25 (39.22%).

Discussion: The study's findings suggest that six PA phages isolated from Kenyan wastewater samples exhibit the broadest host range, making them potentially effective against a significant proportion of clinical isolates. Identifying phages with high productivity against endemic strains is promising for developing a cocktail phage therapy that could be used as an alternative treatment for drug-resistant PA infections. As some endemic strains are not targets of the identified phages, additional phages with a more diverse host range will be identified for more tailored approaches to address the challenge of strain specificity/narrow spectrum in phage therapy.

Conclusion: This study contributes valuable information on the feasibility and limitations of using phages as a therapeutic option for combating multidrug-resistant P. aeruginosa infections in the context of the Kenyan healthcare system.

15. PROFILING OF HUMAN BIOLOGICAL SAMPLES EXPORTED BY KEMRI RESEARCHERS BETWEEN 2018 AND 2020

Authors: Timothy Kipkosgei Kiplagat (KEMRI)*; Gideon Msee (KEMRI); Serah Gitome (KEMRI); Enock Kebenei (KEMRI); Daisy Cheruiyot (KEMRI); Maryanne Metto (KEMRI); Judy Waithera (KEMRI); James Nguya (KEMRI); Geoffrey Sang (KEMRI); Victoria Soi (KEMRI); Cyprian Kisienya (KEMRI); Lilian Achacha (KEMRI); Daisy Mudegu (KEMRI)

Background: Sub-Saharan African countries have recorded increased exportation of human biological samples to foreign high-income countries for analysis and storage. Among the justifications for such export include a need for further analysis of biological samples due to a lack of capacity for analyzing the samples locally. In this study, we profiled the reasons for export, the types of samples exported, and types of tests to be done, and the destinations of the samples exported.

Methods: A data collection tool was developed in MS Excel and abstraction was done from 439 archived SERU records of requests for biological sample export for the period January 2018 to May 2020. We excluded records that did not contain human biological samples and those that were never approved. The period from January 2018 to May 2020 was selected because it had complete records of the profiles of the human biological samples (types of samples, types of tests, reasons for export, destination of export, and duration of storage). R statistical was used to perform data analysis. Frequency distributions, and measures of central tendency (mean, median, and mode) were used to describe profiles of the samples shipped and the type of analysis to be performed outside Kenya. 14th 234 kash Conference

Results: A total of 816 requests for the export of various samples were abstracted from the 439 records. A majority, 613 (75%) were for blood-related samples. Of these, the majority were plasma samples 194 (23.7%), followed by serum samples 136 (16.6%), whole blood samples, 102 (12. %), and 48% (387) included other human tissues and DNA extracts. The most common reason provided for export was further analysis (n=272) with varied assays: Pharmacokinetic/Pharmacodynamics (n=72), Quality assurance and Control (QA/QC) n=58, Immunologic Testing n=48, Endpoints Analysis (n=44), genotyping (n=42, drug level analysis (n=40), molecular analysis (n=38), PCR (n=36), antibody testing (n=30) among others. Most of the requests were for samples being exported to the USA, 327 (53%) followed by South Africa 104 (17%), Switzerland 45(7%) United Kingdom 43(7%) while the remaining n=97 (23 %) we shipped to other countries in Africa, Asia, Australia and Europe.

Conclusion: This study shows that most of the requests for export are to the USA for Pharmacokinetic/Pharmacodynamics, Quality assurance and Control (QA/QC), Immunologic Testing, and Endpoints Analysis. There is a need to build local infrastructural and human resource capacity to run some of the assays in Kenya to minimize the frequent requests for the export of samples where possible.

16. DEVELOPMENT OF A SPECIFIC IMMUNE LATERAL FLOW ASSAY FOR CHOLERA DETECTION

Authors: Freek van't Hoen (TNO)*

Background: Cholera is an acute diarrheal disease caused by Vibrio cholerae, that can be lethal within hours if left untreated. People are usually infected with V. cholerae by using contaminated water or food. UNICEF indicate that every year there are as many as 4 million cholera cases globally and as many as 143,000 deaths. Young children, especially those under the age of 5, are at risk. Rapid detection and identification is essential for the most effective response to an infectious disease outbreak. Therefore, our goal is to develop a more specific Point-of-Care test.

Methods: The following steps were taken for development of a specific Point-of-Care diagnostic test: 1. Determine a specific biomarker of the epidemic V. cholerae strain. 2. Analyze with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) different V. cholerae isolates. 3. Determine if a specific biomarker can be discovered. 4. Develop and test mono-clonal antibodies (mABs) against this specific biomarker. 5.Currently, a prototype immuno-lateral flow assay is being developed. 6. Evaluation and validation of the lateral flow assay.

Results: 1. Cholera is caused by a pandemic strain of Vibrio cholerae. The strain can have a O1 or a O139 serotype and produces the cholera toxin (Figure 1). Other V. cholerae isolates aren't O1 or O139 or are O1 but don't produce the cholera toxin. The complexity is to detect a biomarker that discriminates the pandemic strain from other V. cholerae isolates. 2. Using MALDI-TOF MS a biomarker was found, which is specific for the pandemic V. cholerae strain. Moreover, by using Ferulic Acid as a MALDI matrix a specific protein for the V. cholerae pandemic strain was discovered (Figure 2 and 3). 3. After a band at 35kDa was cut out of a SDS-PAGE gel and analyzed with LC-MS.MS the protein was identified as OmpU. A abundantly expressed outer membrane protein of V. cholerae (Figure 4). 4. Monoclonal antibodies (mABs) were developed and tested. A dotblot confirmed that the mABSs specifically bind to the pandemic V, cholerae strain. 5. The development of the immuno-lateral flow assay is ongoing. After the prototype is developed its performance will be evaluated in a laboratory against different bacteria. Next, if successful, the immuno-lateral flow assay's will be validated in the field, in an area where cholera is endemic.

Conclusion: Using MALDI-TOF MS a specific biomarker for the pandemic V. cholerae strain was detected. The abundantly expressed outer membrane protein OmpU is a promising candidate to develop a imunno-

later-flow assay. Therefore, specific monoclonal antibodies were developed against OpmU. The specific mABs are now used to develop a LFA-prototype. Next, the LFA will be evaluated in the lab and if successful field tests will be conducted in areas where cholera is endemic.

17. ASSESSMENT OF GENETIC DIVERSITY OF P. FALCIPARUM CHONDROITIN SULFATE A LIGAND; A PREGNANCYASSOCIATED MALARIA VACCINE ANTIGEN

Authors: Rotich K Alex (Mt.Kenya University)*; Bernard Kanoi (Mt. Kenya University); Jesse Gitaka (Mount Kenya University); Kevin Mbogo (JKUAT)

Background: Plasmodium falciparum malaria poses a substantial threat to global health, particularly affecting vulnerable populations such as children and pregnant women. Pregnancy-associated malaria (PAM) is primarily driven by the sequestration of infected erythrocytes in deep vascular beds, facilitated by parasite-derived ligands like VAR2CSA. Developing interventions against PAM requires a comprehensive understanding of these mechanisms. This study focused on the genetic analysis of CSA-L, a ligand associated with sequestration, to explore its potential as a vaccine candidate.

Methods: Sampling, DNA preparation, and whole-genome sequencing were conducted on archived whole blood samples from patients enrolled in a drug resistance surveillance study on selected islands in Lake Victoria and a coastal mainland area. The study spanned from July 2014 to July 2016, with a total of 26 samples. Read mapping and coverage involved aligning sequence reads against the Plasmodium falciparum 3D7 reference genome using the Burrows-Wheeler Alignment tool (BWA). Subsequent processing with Samtools and Picard removed duplicates. SNPs were called using the Genome Analysis Toolkit (GATK) HaplotypeCaller. Variant analysis utilized the VCF file containing quality-tested samples. 14th 268 kash Conference Functional annotation of high-quality SNPs in CSA-L was performed using the SNPEFF tool, and ARTEMIS software was employed for further analysis. Multiple sequence alignment and translation of nucleotide sequences to amino acid sequences were conducted using MUSCLE in MEGA 11.

Results: Genetic analysis of CSA-L revealed conservation, supported by Tajima's D of -1.73363 and Pi of 0.00033. Comparative analysis with Var2csa and Pfadsl genes demonstrated CSA-L's higher conservation. Fu & Li's tests indicated significant values for CSA-L, suggesting potential selective pressure. Discussion: The observed negative Tajima's D, Fu and Li's D* and Fu and Li's F* statistics from the Homabay population indicated an excess of rare variants and do not suggest balancing selection (Ndwiga et. al 2021). Genes with a significant negative Tajima's D value indicate that the parasites population has a limited potential to retain polymorphisms, especially Csa-l and Pfadsl gene (Amambua et. al 2012). These findings are consistent with previous studies of P. falciparum in the African population, which showed a majority of genes having a negative Tajima's D value, suggesting a historical parasite population expansion event (Ocholla et. al 2000)

Conclusion: The observed genetic conservation of CSA-L underscores its significance, urging further investigation into its functional relevance as a potential vaccine candidate. Understanding the genetic dynamics of ligands associated with sequestration is crucial for developing targeted interventions against pregnancy-associated malaria

18. TOWARDS THE DEVELOPMENT OF A MICROFLUIDIC DEVICE FOR POINT-OF-CARE DETECTION OF VIABLE CRYPTOCOCCUS NEOFORMANS

Authors: Mary N Wachira (NUITM-KEMRI, Kenyatta University) *

There is a significant diagnostic challenge in differentiating between cryptococcal meningitis (CM) relapse, persistent CM or cryptococcal Immune Reconstitution Inflammatory Syndrome (IRIS), each of which

requires specially tailored therapy. We aimed to develop a microfluidic point-of-care device based on the Immiscible Filtration Assisted by Surface Tension and Adenosine Triphosphate (IFASTATP) assay for detecting viable Cryptococcus neoformans, the causative agent of CM and IRIS, which would enable appropriate and timely clinical treatment.

Introduction: Cryptococcal meningitis (CM) results from haematogenous spread of Cryptococcus neoformans to the brain and meninges once cellular immunity is compromised especially in HIV/AIDS patients. Current diagnostic methods for detecting the causative agent, C. neoformans, are time-consuming, less sensitive and do not reflect viable C. neoformans, hence not suitable for resource-poor areas. Microfluidic lab-on-a-chip technology based on the specific Immiscible Filtration Assisted by Surface Tension (IFAST) and the sensitive Adenosine Triphosphate (ATP) assay offers the potential to address these challenges [1]. Here, we report the progress in the development of a microfluidic device for the rapid point-of-care detection of viable C. neoformans from cerebral spinal fluid based on IFAST and ATP bioluminescence assay.

Results and Discussion: Our concept for IFAST/ATP assay comprises of two steps: (a) immunomagnetic isolation and (b) detection of viable C. neoformans. First, the immunomagnetic isolation of C. neoformans was investigated. Briefly, functionalised magnetic beads specific to C. neoformans were not commercially available, and were therefore prepared in-house. Anti-C. neoformans antibody binding to C. neoformans cells was confirmed by colony count. A 98.4% immunomagnetic binding efficiency was observed from off-chip tubebased conjugation of biotinylated anti-C. neoformans antibody to streptavidin-magnetic beads via streptavidin-biotin reaction at room 14th 280 kash Conference temperature for 30 minutes to afford a final concentration of 10 µg antibody per 1 mg magnetic beads. The cells isolated by C. neoformans functionalized magnetic beads were viable. A 75% immunomagnetic capture efficiency was observed from off-chip tube-based isolation of C. neoformans with functionalized anti-C. neoformans antibody at room temperature with 30µl of 10µg antibody per mg beads. A 10-minute incubation time at room temperature gave the highest capture efficiency of 56.5%. CONCLUSION: A simple and fast system for immunomagnetic detection of C. neoformans has been explored for the first time using IFAST, showing great promise for timely and accurate point-of-care diagnosis of cryptococcal meningitis and cryptococcal immune reconstitution inflammatory syndrome.

19. NEOANTIGEN PROFILING IN KENYAN BREAST CANCER PATIENTS USING WHOLE EXOME AND RNA SEQUENCING

Authors: Godfrey Wagutu (Mount Kenya University); John Gitau (Mount Kenya University); Kennedy Mwangi (Mount Kenya University); Mary Murithi (Kabarak University); Francis W. Makokha (Mount Kenya University) *

Background: Immune response against tumors is dependent on the discrimination between self and non-self. Cancer immunotherapy aims to enhance this anti-tumor response for elimination of cancerous cells. Utilization of neoantigens derived from somatic mutations forms the basis for many cancer immunotherapeutic strategies. Given the heterogeneity of breast cancer, understanding its neoantigen landscape becomes crucial for targeted immunotherapeutic interventions. However, there is a scarcity of such information for specific populations, including Kenya. This poses a challenge in tailoring immunotherapeutic strategies for breast cancer patients in the region. This study's objective was to profile neoantigens in Kenyan breast cancer patients for advancing precision medicine and realizing the full potential of immunotherapy within this population.

Methods: Whole-exome sequencing (WES) and RNA-seq data from tumor-normal matched samples of 23 Kenyan breast cancer patients were used. Somatic mutations were identified from the WES data, while

tumor RNA-seq data was used to quantify the expression of the identified mutations. Neoantigens prediction focused on human leukocyte antigens (HLA) crucial to cancer, HLA type I. HLA alleles were predicted from each patient's normal sample exome-seq data, with four alleles that were present in 50% of the patients selected. Predicted neoantigens were deemed potentially immunogenic if their median IC50 binding scores were ≤500nM and were expressed (Transcripts per million, TPM>1) in tumor samples.

Results: An average of 1465 neoantigens covering 10260 genes had ≤500nM median IC50 binding score and >1 TPM expressed value for the 23 patients, and were significantly correlated with the somatic mutations (R2=0.570, P=0.001). In a panel of 58 genes reported in the catalog of somatic mutations in cancer (COSMIC, v99) to be mutated in breast cancer, 44 (76%) produced >2 neoantigens, with a mean of 10.52 ranging from 2 to 93. For the 44 genes, a total of 477 putative neoantigens were identified, predominantly derived from missense mutations (88%), indels (6%) and frameshift mutations (6%). Notably, 78% of the putative breast cancer neoantigens were patient specific. HLA-C*06:01 allele was associated with majority of neoantigens (194), followed by HLA-A*30:01 (131), HLA-A*02:01 (103), and HLA-B*58:01 (49). Among the genes of interest that produced putative neoantigens include TP53, GATA3, PIK3CA, MAP3K1, BRCA1&2, and ARID1A.

Conclusion: The unique neoantigen profiles highlight the potential of immunotherapy in personalized breast cancer treatment in the Kenyan population. Furthermore, our findings establish a foundation for increased genomic utilization in breast cancer diagnosis and prognosis.

20. FIRST SEROLOGICAL EVIDENCE OF LOUSE BORNE RELAPSING FEVER IN NORTHERN KENYA: A RETROSPECTIVE STUDY

Authors: John Njeru (KEMRI)*

Background: Louse-borne relapsing fever (LBRF) is a vector-borne zoonotic disease transmitted to humans by infected body lice and is associated with significant morbidity and mortality in febrile patients in endemic regions. LBRF is caused by a highly motile spirochete bacterium Borrelia recurrentis. Patients with LBRF present with recurrent high fever and spirochetemia which are accompanied mainly by rigors, headache, dizziness and generalized aches shortly a few days after infection. Frequent outbreaks have been reported in Eritrea, Ethiopia, and South-Sudan mainly in the regions strongly associated with war, famine, poverty, overcrowding and breakdown of personal hygiene. Kenya continues to receive increasing number of refugees from the neighboring countries. Thus, there is a likelihood that refugees may introduce vectors carrying LBRF spirochetes to the communities at the bordering counties of Kenya. Currently, there are no proper epidemiologic data available on the burden of LBRF in Kenya. This study aimed to assess the seroprevalence of LBRF in samples of febrile patients of all ages at the selected hospitals in the Turkana County collected between 2009 and 2010.

Methods: A total of 2,030 Bio-banked, frozen (-80oC) serum samples were thawed to room temperature and screened for the presence of IgG and IgM antibodies against Borrelia recurrentis using a recently validated IgG and IgM based iELISA by our group for serodiagnosis of LBRF.

Results: Overall, 415(20.5%) samples were found to be seropositive for LBRF fever based on a parallel interpretation of the IgG and IgM tests applied. Of these, 328(16.2%) had IgG antibodies while 87(4.3 %) had IgM. An additional 160(7.91%) and 17(0.8%) samples had borderline IgG and IgM antibodies respectively suggesting past exposure. Sixty eight percent of the positive samples were from patients residing from Turkana North (224/328) while 103(31.4% were from Turkana East. Only one sample tested positive among samples from Turkana Central and none from Turkana west. Male accounted for 966(47.6%) of the participants. Of these 153/966 (15.8%) tested positive for LBRF. A total of

175/1064(16.4%) women tested positive for LBRF. Majority (93/328) of positive samples were from participants aged 31-40 years followed by those aged between 41-50 years (79/328).

Conclusions: Our findings provide the first evidence of the presence of LBRF as a serious public health problem in northern Kenya. Considering that LBLF is not systematically considered during routine diagnoses of febrile illnesses in Kenya, due to lack of simple diagnostic assays, such patients often encounter missed opportunities for accurate detection and treatment of their infection. There is therefore a critical need for the deployment of public health awareness about the disease and appropriate management guideline and outbreak preparedness in Kenya. Keywords: Serological,Louse borne relapsing fever,Northern Kenya

21. ANTIMICROBIAL SUSCEPTIBILITY AND GENETIC BASIS OF RESISTANCE OF KLEBSIELLA SPP ISOLATED FROM DIARRHEIC AND NON-DIARRHEIC CHILDREN AT HEALTH FACILITIES IN MUKURU INFORMAL SETTLEMENT, NAIROBI, KENYA

Authors: celestine w wairimu (Kenya Medical Research institute) *; Samuel Kariuki (KEMRI)

Background: Antimicrobial resistance (AMR) is a global threat to public health and particularly to children. This study aimed to determine the prevalence of multidrug resistance of fecal Klebsiella spp on selected beta-lactam (3rd generation cephalosporins and carbapenems) and fluoroquinolone classes of drugs in four health facilities serving the Mukuru slum community of Nairobi city in Kenya. Additionally, to determine the genetic basis for the multidrug resistance observed.

Methodology: A cross-sectional laboratory-based study was undertaken where a total of 1171 children below 16 years were selected, from whom stool samples were collected, tested, and analyzed using various microbiological methods namely; culture, biochemical testing, antibiotic sensitivity testing and polymerase chain reaction. A total of 395 (33.73%) Klebsiella spp were isolated, consisting of 365 (92.4%) Klebsiella pneumoniae and 30 (7.6%) Klebsiella oxytoca were isolated.

The proportion of multi-drug resistance (MDR) K. pneumoniae and MDR K. oxytoca was 64.1 % (234/365) and 96.67 % (29/30) respectively. MDR was defined as was defined as an isolate nonsusceptible to at least one agent in three or more antibiotic classes K. pneumoniae showed the highest resistance against third-generation cephalosporins namely; cefotaxime 112 (30.7%), ceftriaxone 109 (29.9%), and ceftazidime 100 (27.4%), whereas the least resistance was observed against carbapenems including imipenem 6 (1.6%) and meropenem 6 (1.6%). Out of 365 K. pneumoniae, 42 (11.5%) showed resistance to both third generation cephalosporins and fluoroquinolones. A significant association was observed in diarrheic children (OR =1.88; p=0.01) and those below 50 months (OR = 0.43; p=0.002) and carrying K. pneumoniae resistance to one or more third-generation cephalosporins. Among the K. pneumoniae isolates resistant to both third generation cephalosporins and fluoroquinolones, resistance genes identified included bla TEM 42 (100%), bla CTX-M 40 (95.2%), bla SHV 24 (57.1%), bla OXA-1 28 (66.7%), qnrS 23 (54.1%), qnrB 20 (47.6%) and bla NDM 3 (7.1%).

In conclusion, there is a high prevalence of MDR K. pneumoniae carrying genes associated with antibiotic resistance, and this poses a threat to the Mukuru community, especially the vulnerable populations.

22. TARGETING MYCOBACTERIUM TUBERCULOSIS WITH MYCOBACTERIOPHAGE

Authors: Joseph Gitari (University of Cape Town) *; Elizabeth Kigondu (KEMRI); Mandy Mason (University of Cape Town); Anastasia Koch (University of Cape Town); Digby Warner (University of Cape Town)

The survival of Mycobacterium tuberculosis in dynamic, often hostile, microenvironments during host infection is thought to be partly attributable to the bacillus's capacity for heterogeneity. This trait manifests, too, in the existence of genetically susceptible but antibiotic recalcitrant M. tuberculosis subpopulations – variously referred to as antibiotic tolerant, persistent, or resilient cells which have been implicated in treatment prolongation, and relapse following non-adherence to therapy. There is growing interest, therefore, in the development of innovative interventions to increase the efficiency of M. tuberculosis clearance, including through the application of biologics such as engineered mycobacteriophages.

This study utilizes the lytic mycobacteriophage D29 to investigate the disruption of the complex mycobacterial cell wall to enhance antibiotic efficacy and mycobacterial lysis. We use live-cell time-lapse imaging and flow cytometry to demonstrate phage-mediated lysis and antibiotic uptake in non-pathogenic M. tuberculosis (H37Ra) and M. smegmatis models.

We have determined the efficiency of mycobacteriophage D29 in enhancing mycobacterial cell lysis alone in non-pathogenic M. tuberculosis (H37Ra) and rifampicin-resistant M. smegmatis models, and in combination with cell wall-targeting antimycobacterial drugs in wildtype M. smegmatis. In flow cytometry and live-cell time-lapse microscopy assays utilizing fluorescent M. smegmatis bioreporter mutants, we have demonstrated phage-mediated lysis of single mycobacterial cells under microfluidic culture. Phage adsorption was observed at the poles and septa of actively replicating bacilli, consistent with the known involvement of these sites in the addition of new cell wall material. Notably, phage lysis reduced mycobacterial survival to <0.0001% for all strains tested, with statistically significant differences (p<0.001), supporting the efficiency of phage independent of genetic drug susceptibility. In ongoing work, we are investigating the use of D29 mycobacteriophage as an adjunct to conventional antibiotic treatment to enhance killing of antibiotic recalcitrant mycobacterial cells

23. ISOLATION AND CHARACTERIZATION OF BACTERIOPHAGES WITH LYTIC ACTIVITY AGAINST DRUG-RESISTANT NON-TYPHOIDAL SALMONELLA FROM NAIROBI CITY COUNTY, KENYA

Authors: Michael Mugo1,2*, Abednego Musyoki2, Angela Makumi3, Ivy Mutai4, Kelvin Kering1, Peter Muturi1, Cecilia Mbae1, Samuel Kariuki1 1. Centre for Microbiology Research, Kenya Medical Research Institute, Nairobi, Kenya. 2.Department of Medical Laboratory Science, Kenyatta University 3. International Livestock Research Institute (ILRI) 14th 298 kash Conference 4. Phage Biology Laboratory, Institute of Primate Research, Nairobi, Kenya *Corresponding author: mikemugom@gmail.com

Background: Whereas Non-typhoidal Salmonella (NTS) is a common cause of self-limiting enterocolitis in humans, it has been implicated in life-threatening invasive cases. Emergence of Multidrug-resistant NTS has been reported in Kenya with confirmed cases of resistance to 3rd generation cephalosporin. Due to the emergence of multi-drug resistant (MDR) bacteria coupled with the slowed development pace of new antimicrobial agents, bacteriophages are considered as a feasible alternative to antimicrobials.

Methods: This study, therefore aimed to isolate phages that can be used as an alternative therapy against MDR NTS infections. In vitro assessment of phage efficacy was conducted by host range and efficiency of plating (EOP) assay, using a panel of twelve Salmonella isolates. Phages with the broad host range were selected to determine their efficiency using EOP assay. Later, their physiochemical properties were determined by assessing phage thermal and pH stability. The effects of phages on NTS biofilm was tested by introducing phages on already formed NTS biofilms.

Results: Thirty-one phages were isolated from environmental samples collected within Nairobi city county. They exhibited a narrow to broad host range (8%-100%), with ten phages lysing 80% of the Salmonella

strains used for the host range. The ten phages were selected for EOP and physio-chemical characterisation. All phages showed high production efficiency in at least one isolate which was not of their host, apart from two phages. All phages demonstrated relative stability between -80ºC to 40ºC temperature range, with a slight titre reduction at 50ºC for some phages. Relative stability was also observed at pH 5 to 11 with the highest titres recorded at pH 7 to 9. Phages also demonstrated a significant ability to digest NTS biofilms.

Conclusion: This study presents the availability of potential phage-therapy candidates from Nairobi city county, which can be applied against MDR NTS. The Phages lytic activity with broad pH and temperature stability, and biofilm digestion indicates their potential in therapeutic purposes.

24. MISSED OPPORTUNITIES FOR DATA USE IN HEALTH CARE DECISION-MAKING IN KENYA: CROSS SECTIONAL DIGITAL HEALTH LANDSCAPE ASSESSMENT. (This can help in the UAT project)

Authors: Mercy Terer (KEMRI-CGHR) *; Miriam Taegtmeyer (Liverpool School of Tropical Medicine) Chepkirui M1,3, Musuva R2, Dellicour S3, Akoth I1, Omondi B1, Barsosio H1, Otiso L2, Okomo G6, Tancred T3, Alhassan Y3, Waweru M4, ter Kuile F1,3, Lesosky M5, Kariuki S1, Taegtmeyer M3 1. Malaria Branch, KEMRI-Center for Global Health Research (CGHR) 2. LVCT Health 3. Liverpool School of Tropical Medicine 4. GIND 5. Imperial College, London, UK 6. Department of Health, Homabay county

Background: Sub-Saharan Countries have a rising uptake of eHealth and mHealth interventions to improve data quality, decisionmaking, and accelerate healthcare access in the last decade. The proliferation of digital health solutions has led to an e-chaotic situation with electronic medical records (EMR) systems implementation characterized by fragmented implementation, inadequate system linkages, limited data sharing, and reliance on short-term, donor-driven funding. Consequently, there is an exponential growth in health data generation that is underutilized for decision-making, patient-centric care, and research.

Methods: A cross-sectional landscape assessment was carried out in Homabay County in Kenya between June 2022 and Oct 2023 in 113 public health facilities. We used health facility surveys and 33 in-depth interviews with relevant health system stakeholders to map what EMRs were present (and functioning) and explored perspectives on the utility of EMRs for data use in decision-making.

Results: Nine unique EMR systems were identified across the surveyed facilities (1 inactive). Ninety-two percent (104/113) of the health facilities surveyed had KeEMR used for HIV patient care. Seven additional EMR systems (WebADT, USHAURI, Elephant, LIMS, WISECARE, CAREPOINT & FANSOFT) were identified in active use alongside KeEMR. Five out of the eight active EMRs were supported by implementing partners and faith-based organizations. EMR challenges were lack of interoperability, internet connectivity, system downtime, patient workload, power outage, staff turnover, and lack of universal patient identifiers. Data quality checks, reporting, and data reviews were done routinely in 92% of the facilities.

Conclusion: The study findings mirror digital health systems utilization in public health care settings. Despite the heavy skewness towards HIV care and lack of interoperability, there is a huge potential for effective utilization of EMR in low-resource settings. There is a need for the government to take a central leadership in driving EMR implementation, defining scope and purpose to cover the general population. Implementation of a sustainable patient record identification mechanism will allow data sharing amongst platforms and promote inter and intra-facility longitudinal patient follow-up. A centralized secure platform for data access will further facilitate data uptake and use for public health research and decision-making. A journey that will involve developing standardized data quality protocol to enable good quality data use for health care decision making