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Flies as carriers of antimicrobial resistant (AMR) bacteria in Nigerian hospitals: A workflow for surveillance of AMR bacteria carried by arthropod pests in hospital settings

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ABSTRACT

The dissemination of antimicrobial resistant (AMR) bacteria by flies in hospitals is concerning as nosocomial AMR infections pose a significant threat to public health. This threat is compounded in low- and middle-income countries (LMICs) by several factors, including limited resources for sufficient infection prevention and control (IPC) practices and high numbers of flies in tropical climates. In this pilot study, 1,396 flies were collected between August and September 2022 from eight tertiary care hospitals in six cities (Abuja, Enugu, Kaduna, Kano, Lagos and Sokoto) in Nigeria. Flies were screened via microbiological culture and bacterial isolates were phenotypically and genetically characterised to determine carriage of clinically important antibiotic resistance genes (ARGs). Several clinically relevant ARGs were found in bacteria isolated from flies across all hospitals. bla_{NDM} was detected in 8% of flies and was predominantly carried by *Providencia* spp. alongside clinically relevant *Enterobacter* spp, *Escherichia coli* and *Klebsiella pneumoniae* isolates, which all exhibited a multidrug resistant phenotype. *mecA* was detected at a prevalence of 6.4%, mostly in coagulase-negative Staphylococci (CoNS) as well as some *Staphylococcus aureus*, of which 86.8% were multidrug resistant. 40% of flies carried bacteria with at least one of the two ESBL genes tested (bla_{OXA-1} and $bla_{CTX-M-15}$). This multi-site study emphasised that flies in hospital settings carry bacteria that are resistant to multiple classes of antibiotics,

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including both routinely used and reserve antibiotics. A greater understanding of the global clinical significance and burden of AMR attributable to insect pests is required.

1. Introduction

Antimicrobial resistance (AMR) is a global crisis affecting multiple facets of human life, from food security and global economies, to increasing mortality and morbidity caused by drug-resistant infections (DRIs) (Levy and Marshall, 2004). The 'one health' approach to AMR mitigation requires an understanding of the dynamics between human, animal and environmental systems (Djordjevic, 2024). In clinical settings, the spread of AMR is a major concern as it results in limited treatment options for patients with DRIs, with attendant increases in morbidity, mortality and cost of care. AMR emerges in hospitals due to multiple factors, including patients with community-acquired AMR infections, close contact with environmental reservoirs of AMR and inappropriate use of antimicrobial products (Chokshi et al., 2019). In LMICs, the burden of AMR is exacerbated by limited resources available for diagnostics, surveillance, infection prevention and control (IPC) and appropriate antibiotics for the treatment of DRIs within hospitals.

Additionally, many LMICs in the tropical region have optimal climates for the presence of fly populations, as average temperatures often align with housefly thermal preferences (25–34 °C) (Delclos, 2021). The presence of flies in hospital settings is further enhanced by inadequate hospital infrastructure, waste management, and lack of pest control resources. Whilst various arthropods act as carriers of AMR pathogens (Davari et al., 2010; Gwenzi, 2021; Hassan et al., 2021; Laroche et al., 2018; Odetoyin et al., 2020), flies are of particular concern due to their vast abundance and synanthropic nature. The involvement of flies in the environmental AMR landscape has been demonstrated in various locations, including clinical settings, residential dwellings, food production and preparation areas, farms, and open markets (Gwenzi, 2021; Rahuma et al., 2005; Blazar et al., 2011; Lindeberg, 2018; Dehghani and Kassiri, 2020; Jian, 2021; Neupane et al., 2020; Sandrasaigaran, 2023). Houseflies and other species belonging to the *Musca* genus are some of the most abundant flies commonly observed in hospital settings (Gwenzi, 2021; Lindeberg, 2018; Barro et al., 2006; Boiocchi et al., 2019). They have the propensity to thrive in areas of high bacterial load, such as food, domestic and animal waste, and sewage systems (De Jesús et al., 2004; Barro et al., 2006; Schaumburg, 2016; Onwugamba et al., 2020; Yin et al., 2022). Flies act as mechanical transmitters of AMR bacteria through regurgitation, defecation and shedding from the bodily surface (mainly proboscis and legs) (Gwenzi, 2021). Their unique biology, ubiquitous nature, and ability to travel between 5–7 km (Onwugamba et al., 2020) make them feasible propagators of AMR bacteria (Yin et al., 2022). In addition, flies can act as vectors of bacterial disease outbreaks, including enterohemorrhagic colitis (Agui, 1999); shigellosis (Levine and Levine, 1991) and trachoma (Selby et al., 2023) caused by *Escherichia coli, Shigella* spp., and *Chlamydia trachomatis* respectively. Despite increasing evidence, flies are generally overlooked as IPC targets in hospital settings, and as valid contributors to AMR burden in general (Nayduch et al., 2023).

AMR is predicated by the spread of antimicrobial resistance genes (ARGs) (Jian, 2021). Horizontal gene transfer (HGT) via conjugative plasmids enables ARGs to spread within and between species in bacterial communities. Clinical settings are often hotspots for the spread of ARGs in this way, with 'multispecies clusters' of ARGs often being observed (Arcari, 2020). The importation of AMR bacteria from natural environmental sources, which can have a greater diversity of ARGs (Martínez, 2008), is another means of introduction into a clinical setting, especially if these bacterial communities overlap at some point in time or space (Perry and Wright, 2013). Flies may act as a 'shared niche' for environmental and clinical bacteria, or as hotspots for the exchange of ARGs. Their mobile nature may allow them to play a greater role in the dissemination of AMR between different settings than has been previously understood. Flies carrying AMR bacteria with similar genotypes have been identified in humans and animals in overlapping locations, including both urban and hospital settings (Hassan et al., 2021; Onwugamba et al., 2020), indicating a serious public health concern.

The aim of the *Arthropods as Vectors of Infection and Antimicrobial Resistance* (AVIAR) study is to understand the role of flies in contributing to networks of pathogenic bacteria and AMR clusters in hospital settings. This AVIAR pilot study was conducted to gather a baseline dataset assessing the prevalence of ARGs from flies across Nigerian hospitals, and to develop a microbiological procedure for the characterisation of AMR bacteria harboured by flies. This study will enable the development of a large, international project on the prevalence and diversity of AMR bacteria harboured by flies across varied climates and countries; not least comparing high-income countries and LMICs. In this pilot study, bacteria cultured from flies collected from eight hospitals across Nigeria were screened for ARGs and bacterial isolates were profiled through antimicrobial susceptibility testing (AST) and whole-genome sequencing (WGS).

2. Methods

2.1. Study design and fly collection

The study was conducted in eight hospitals in Nigeria – Ahmadu Bello University Teaching Hospital (ABUTH), Zaria, Aminu Kano Teaching Hospital (AKTH), Kano, Barau Dikko Teaching Hospital (BDTH), Kaduna, Lagos University Teaching Hospital (LUTH), Lagos, Murtala Mohammed Specialist Hospital (MMSH), Kano, National Hospital Abuja (NHA), University of Nigeria Teaching Hospital (UNTH), Enugu, and Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto. These hospitals, which are located across six Nigerian states, were selected based on regional coverage and existing collaborations (Global surveillance of AMR). The hospitals had bed capacities ranging from 300 to 950 patients and were mostly located in urban settings (Table 1).

Material Transfer Agreements (MTAs) were completed prior to the study and fly collection packs (Supplementary Table 1) were shipped to the hospitals. Hospital wards were selected for sample collection based on ease of access of placing fly traps and likelihoods of staff members to comply with the study methods. Environmental data regarding the surrounding vicinities, and clinical antibiotic usage data were collected for each site via a questionnaire (Tables 1 & 2). In addition, each site completed a post-collection survey to provide feedback on the effectiveness of the methods used, which will be used to inform and refine the AVIAR multinational study.

Flies were collected between the 15th of August 2022 and the 20th of September 2022. Sticky Fly Traps (SFTs, Pelsis UK, Fixman, UK) were placed in hospital wards (Table 2 & Supplementary Table 1). Metadata was recorded for each SFT placed, including ward location (What3-Words, UK – a software application readily available providing unique three-word combinations for a 3-metre location to enhance accuracy and exact locations), date of SFT placement and removal, and temperature and relative humidity which were recorded using a temperature and humidity sensor (RS PRO TA298 Hygrometer). SFTs were checked daily, and trapped flies were transferred into labelled 7 mL bijous after each day of collection. Qualitative descriptions of fly species were provided based on a graphical Dipteran morphological identification key adapted from Parveen's 'The Wonders of Diptera' (Perveen et al., 2021). Flies were stored at 4 °C prior to shipment to the University of Oxford,

UK under UN3373 conditions for microbiological processing. Flies were shipped from each hospital site after the collection period for that hospital was complete.

2.2. Microbiological processing of flies

Flies were individually homogenised in 1 mL of 0.85 % (physiological) saline for 20 s at 6.5 m/s using a homogeniser (FastPrep-24™, MP Biomedicals). 50 μL of the fly suspension was inoculated and streaked onto six selective chromogenic agar plates (pre-poured products from Liofilchem®, Italy) (Fig. 1) containing: vancomycin (10 mg/L) (V) for the selective growth of Gram-negative bacteria (GNB), vancomycin and cefotaxime (10 & 1 mg/L) (VC) for the detection of ESBL genes, vancomycin and ertapenem (10 & 2 mg/L) (VE) for the detection of carbapenemase ARGs, MRSA agar for the detection of mec genes, colistin agar (2 mg/L) (COL) for the detection of mobile colistin resistance (mcr) genes and tigecycline agar (0.5 mg/L) (T) for the detection of *tet*(X4) (Merck, Germany). These ARGs were investigated due to their clinical significance, the increased mobility of *tetX* and mcr genes, as well as increased cephalosporin resistance reported in clinical settings.

All agar plates were incubated for 24-hours at 37 °C followed by 24-hours at room temperature, then sweeps of the mixed bacterial culture from VC, VE, MRSA, COL, and T plates were screened for the presence of 15 ARGs by PCR. Primers and PCR reactions were developed to screen for globally prevalent ARGs, including a multiplex PCR reaction to screen for $bla_{\text{CTX-M-15}}$ and $bla_{\text{OXA-1}}$ ESBL genes, a reaction to screen for *mcr*-1,3,8,9,10 variants, a multiplex reaction to screen for *mecA* and *mecC* and a singleplex PCR designed to screen for tetX4 (Supplementary Table 2). bla_{NDM}, bla_{KPC} and $bla_{\text{OXA-48-like}}$ variants were detected using a multiplex reaction with primers previously designed to screen for these carbapenemase genes (Hassan et al, 2021). ARG-positive cultures positive were subject to purity plating to identify the isolate carrying the specific ARG for all except ESBL genes ($bla_{\text{CTX-M-15}}$; $bla_{\text{OXA-1}}$) due to high positivity rates. A second PCR test for each morphologically distinct colony isolated from the positive mixed sample was performed against the target gene. Identification of ARG-positive bacterial isolates was determined using MALDI-TOF MS (Bruker Daltonik, GmbH, UK). Positive isolates were then subject to antimicrobial susceptibility testing and whole genome sequencing (WGS).

2.3. Antimicrobial susceptibility testing

Minimum inhibitory concentrations (MICs) of 16 antibiotics for GNB isolates and 15 antibiotics for Gram-positive bacteria (GPB) isolates were determined for ARG positive bacterial isolates by agar or broth microdilution (Andrews, 2001). Briefly, isolates were incubated for 18 h at 37 °C on agar or in broth with the addition of serially diluted antibiotics to determine the lowest concentration at which growth was inhibited per antibiotic (Supplementary Table 3). *Proteus* and *Providencia* isolates were tested via broth microdilution due to their swarming nature, in cation-adjusted Meuller Hinton broth (Merck, Germany) with antibiotic concentrations ranging from 0.06 to 32 mg/L. All other isolates were tested via agar dilution using Mueller Hinton agar (Merck, Germany) with antibiotic concentrations ranging from 0.06 to 128 mg/L. Seven GNB and two GPB isolates were not tested for MICs due to inability to recover isolates from −80 °C storage. Quality control strains ATCC 25922 *E. coli,* NCTC 13846 *E. coli,* and ATCC 700603 *Klebsiella quasipneumoniae* were used for GNB MIC tests, and ATCC 29213 *Staphylococcus aureus* was used for GPB MIC tests. Results were interpreted as per European Committee on Antimicrobial Susceptibility Testing (EUCAST) v14 (2024) guidelines. The percentage of resistant isolates did not include susceptible increased exposure (Meylan and Guery, 2020). Isolates were considered multidrug resistant (MDR) if they were non-susceptible to at least one antibiotic in three or more antibiotic classes to which they were not intrinsically resistant (Magiorakos, 2012).

2.4. Whole genome sequencing

Isolates that tested positive for any of the target ARGs except ESBLs during PCR screening were selected for WGS. gDNA was extracted from LB broth-cultured bacterial pellets using a QIAamp DNA Mini kit (Qiagen, Germany) with an added RNAse step on a QIAcube connect (Qiagen, Germany). For GPB isolates, a pre-extraction cell membrane lysis step was conducted, whereby pellets were manually resuspended in 60 μL of 10 mg/μL lysozyme and incubated at 37 °C for an hour before being re-pelleted for extraction. Extracted gDNA was quantified using a Qubit Flex Fluorometer 4 with the dsDNA HS assay kit (Life Technologies, USA). Both short read and long read sequencing were performed. Long read sequencing libraries were prepared using the Oxford Nanopore Technologies (ONT) SQK-RBK114.96 Rapid Barcoding Kit (ONT, UK) and sequenced using R10.4.1 flow cells for 72 h on a GridION device (Oxford Nanopore Technologies, UK) with real-time basecalling performed by Guppy v6.4.6. Paired-end short read sequences (300 bp read lengths, v3 chemistry) were generated using the Nextera XT V2 library preparation kit and sequenced on an Illumina MiSeq (Illumina, USA).

Table 1
Study site metadata gained from questionnaires sent to collaborators at each site.

State	Hospital	what3words	Beds	Rural or urban	Close to dump	Close to abattoir	Close to water	Common fly species
Federal Capital Territory, Abuja	National Hospital Abuja	///domesticated.deceived.heaters	450	Urban	No	No	No	Musca domestica
Kaduna State	Ahmadu Bello University Teaching Hospital, Shika-Zaria	///clinicians.admitted.keeping	775	Urban	Yes, 7 km	Yes, 4 km	Yes, 2 km	Musca domestica
Kaduna State	Barau Dikko Teaching Hospital	///clouds.talent.duplicate	300	Urban	No	No	No	Musca domestica
Enugu state	University of Nigeria Teaching Hospital, Ituku-Ozalla	///catacombs.emporium.jumped	550	Rural	No	No	Yes, 0.9 km	Musca domestica
Kano State	Aminu Kano Teaching Hospital	///hometown.maybe.ships	700	Urban	Yes, 1.2 km	No	Yes, 1.2 km	Musca domestica
Kano State	Murtala Muhammad Specialist Hospital	///fruity.magnetic/pickle	1000	Urban	No	No	No	Musca domestica
Lagos State	Lagos University Teaching Hospital	///sliding.tailed.coconut	950	Urban	No	No	Storm drainage canal inside hospital compound	Musca domestica
Sokoto State	Usmanu Dandodiyo University Teaching Hospital Sokoto	///questions.bibs.processor	758	Urban	No	No	No	Musca domestica

2.5. Bioinformatics

Short reads were trimmed using TrimGalore (Krueger, 2024) (v0.6.4) and long reads were trimmed using Filtlong (Wick, 2024) (v0.2.0). Unicycler (Wick et al., 2017) (v0.5.0) was used for hybrid assemblies. For isolates with insufficient long read depth (<20x coverage), short reads were assembled into contigs using shovill (Seemann, 2024) (v1.0.0) and flye (Kolmogorov et al., 2019) (v2.9) was used to assemble long reads with insufficient short read depth. Quast (Gurevich et al., 2013) v5.2.0 was used to generate the assembly quality metrics, and assemblies with a total genome length ≥ 10 % of the expected genome range were excluded. Similarly, genomes with > 400 contigs were excluded. The speciator (v4.0.0) tool within PathogenWatch (Pathogenwatch) (v21.4.3) was used to determine species identification. As RefSeq/bacsort databases are currently limited in terms of representing the recently discovered diversity within the *Providencia* genus (Kolmogorov et al., 2019), assembled *Providencia* genomes were subject to ANI analysis using fastANI (Jain et al., 2018) (v1.33), against the following species reference genomes: *Providencia stuartii* (GCA_029075745.1), *Providencia manganoxydans* (GCA_016618195.1), *Providencia burhodogranariea* (GCA_000314895.2), *Providencia hangzhouensis* (GCA_029193595.2), *Providencia rettgeri* (GCA_900455085.1), *Providencia huaxiensis* (GCA_002843235.3), *Providencia alcalifaciens* (GCA_002393505.1), *Providencia heimbachae* (GCA_900475855.1), *Providencia vermicola* (GCA_020381325.1), *Providencia zhijiangensis* (GCA_030315915.1), *Providencia sneebia* (GCA_000314895.2) and *Providencia rustigianii* (GCA_900455105.1).

ABRicate (Seemann, 2024) (v1.0.1) (database updated April 2024)

Table 2
Metadata for hospital wards, including the number of flies collected per ward, the temperature and humidity ranges during the collection period and a survey of the commonly prescribed antibiotics.

Site	Ward	*n* = *flies*	Temperature Range (°C)	Relative Humidity (%)	Commonly used antibiotics
ABUTH	Female Medical	17	25.9–30	62–70	Ceftriaxone, Ciprofloxacin, Amikacin
	Male Medical	1	27–28.9	60–68	Ceftriaxone, Ciprofloxacin, Metronidazole
	Paediatric Surgical	210	27.4–35	64–70	Clindamycin, Levofloxacin, Cephalosporins, Amoxycillin-Clavulanic acid, Cefuroxime, Kanamycin, Gentamicin, Metronidazole, Ceftriaxone
	Total/average	***228***	***28.5***	***67***	
AKTH	Emergency Paediatric	1	30.7	67	Ceftriaxone
	Female Medical	22	28.5–30	63–67	Ceftriaxone
	Gynaecology	1	27.2	63	Amoxicillin-Clavulanic acid
	Male Medical	18	30.2	61	Ceftriaxone
	Post Natal	13	25.8–28.4	57–66	Amoxicillin-Clavulanic Acid
	Paediatric	1	26.9	57	Ceftriaxone
	Male orthopaedic	0	26.4	69	Ceftriaxone/sulbactam
	Paediatric surgical	0	29.8	68	Ceftriaxone
	Total/average	***56***	***28.4***	***63.8***	
BDTH	Accident & Emergency	5	27.4–28	70–77	Amoxicillin-Clavulanic Acid, Ceftriaxone, Ciprofloxacin
	Paediatric	78	27–28.8	67–72	Penicillin V, Amoxicillin-Clavulanic Acid, Cefuroxime, Ceftriaxone
	Maternity	0	27	69	Amoxicillin- clavulanic acid, Ceftriaxone, Ciprofloxacin
	Medical	0	27	70	Ceftriaxone, Ciprofloxacin
	Surgical	0	27	70	Ceftriaxone, Metronidazole
	Total/average	***83***	***27.5***	***70.8***	
LUTH	Intensive Care Unit	1	27.3	70	Ceftriaxone
	Neurology	32	26.2–27.7	69–78	Ceftriaxone
	Surgery	1	27.1	68	Metronidazole
	Total/average	***34***	***28.2***	***72.2***	
MMSH	Accident & Emergency	6	32.8	57	NA
	Gynaecology	26	32.1	55	Amoxicillin-Clavulanic Acid
	Male Orthopaedic	61	25.1–31.7	57	Ceftriaxone
	Paediatric Orthopaedic	31	31.2	58	Ceftriaxone
	Special Care Baby Unit	35	31–31.9	61–62	Amoxicillin, Chloramphenicol, Gentamicin
	Total/average	***159***	***30.8***	***58.1***	
NHA	Accident & Emergency Medicine	56	24.2–32.6	57–74	Ceftriaxone
	Accident & Emergency Surgery	17	26.3–30.8	60–74	Ceftriaxone
	Neonatal Intensive Care Unit	32	22.7–29.2	62–73	Amoxicillin-Clavulanic Acid
	Orthopaedic	1	28.2	64	Ceftriaxone
	Total/average	***106***	***27.7***	***65.4***	
UDUTH	Accident & Emergency	95	27.1–30	72	Ceftriaxone
	Gynaecology	76	30–31	70–71	Ceftriaxone
	Isolation	7	30.3–31	68–69	Ceftriaxone
	Male Medical	5	27.5–28.7	69–70	Ciprofloxacin
	Neurosurgery	143	26–30.3	68–70	Moxifloxacin
	Paediatric	196	26–31	69–72	Ceftriaxone
	Postnatal	6	27.3–28.1	69–70	Ceftriaxone
	Total/average	***528***	***27.5***	***70***	
UNTH	Female Surgical	5	27.7–28.5	74–76	Meropenem
	Gynaecology	95	27.6–27.9	75–76	Ceftriaxone
	Male Medical	21	26.8	75	Ceftriaxone
	Male Surgical	43	26.8–30.6	63–75	Ceftriaxone
	Oncology	3	27.9	74	Ceftriaxone
	Orthopaedic	13	28.2	70	Ceftriaxone
	Paediatric	22	27.2	79	Ceftriaxone
	Total/average	***202***	***28.2***	***72***	

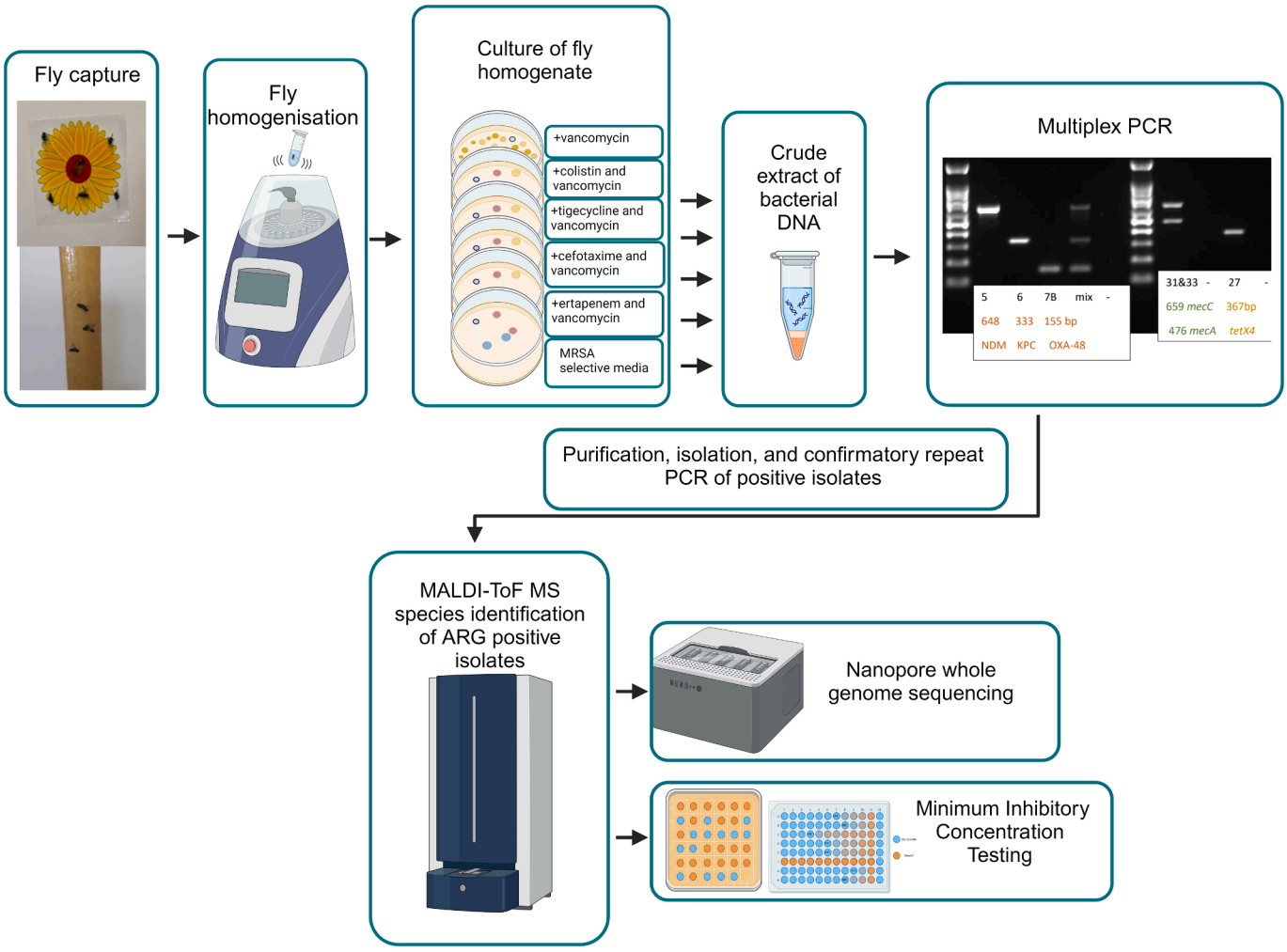

Fig. 1. Graphical workflow of the methodology for the surveillance of AMR bacteria in flies. A combination of microbiological and molecular approaches were utilised to understand bacterial identification, genotyping and genomic context of AMR. Created in BioRender – Premchand-Branker, S. (2024) BioRender.com/k49r379.

was used to screen for ARGs using Resfinder (Florensa et al., 2022). Plasmid incompatibility typing was performed using mob_typer within MOB-suite (Robertson and Nash, 2018) (v.3.1.8). Multi-locus sequence typing (MLST) was determined using *mlst* (Seemann) (v2.23.0) and PubMLST for *Providencia*. Novel alleles and MLST profiles were submitted to PubMLST (PubMLST) for assignment. For *Staphylococcus aureus* isolates; the SCC*mec* cassette was typed using SCCmecFinder (Kaya, et al., 2018) v1.2. Bacterial genomes were annotated using Prokka (Prokka) (v1.15.5) and core genome alignments (0.98 core threshold for species and 0.90 for genera) were created using Panaroo (Tonkin-Hill, 2020) (v1.2.8). IQ-TREE (Nguyen et al., 2015) (v2.0-rc1) was used to generate a maximum likelihood phylogenetic tree with 1000 replicates for ultrafast bootstrap and were annotated using iTOL (Letunic and Bork, 2024) (v6.8.1).

2.6. Statistical analysis

Z-tests for proportional differences in ARG presence and absence between the hospitals were conducted incorporating the Bonferroni correction to adjust for multiple comparisons. Confidence intervals were calculated to account for variations in sample size. Weighted linear regression models were generated to assess for relationships between ARG prevalence, temperature and humidity. Pearson's correlations were conducted to assess for individual associations of temperature and humidity with ARG prevalence. All statistical analyses were conducted in R.

3. Results

3.1. Metadata and bacterial growth from flies

A total of 1,396 flies were collected between August and September 2022 from across the eight hospital sites in Nigeria (Fig. 2, Table 2), with variations in the numbers of flies collected between hospitals and wards (Table 2). Across the hospital sites, the highest number of flies were collected from paediatric wards (n = 537), accounting for over a third of the total, followed by gynaecology (n = 198) and accident and emergency (n = 178) wards. Most flies were identified as belonging to the *Muscidae* family (houseflies) and a small proportion were identified as *Calliphoridae* (blowflies) and *Sarcophagidae* (fleshflies). Over the sampling period, MMSH in Kano had the highest average temperature (30.8 °C) and lowest average humidity (58.1 %) from which 159 flies were collected, whilst BDTH in Kaduna had the lowest average temperature (27.5 °C) and the second highest average humidity (70.8 %), from which 83 flies were collected. Temperatures ranged between 24.2 – 35.0 °C across the sites and humidity ranged from 55-78 % (Table 2). Significant relationships were not observed between temperature or humidity and ARG prevalence (p = >0.05). Ceftriaxone, a third-generation cephalosporin, was the most commonly reported antibiotic used in all hospitals, whilst carbapenem use (in the form of meropenem)

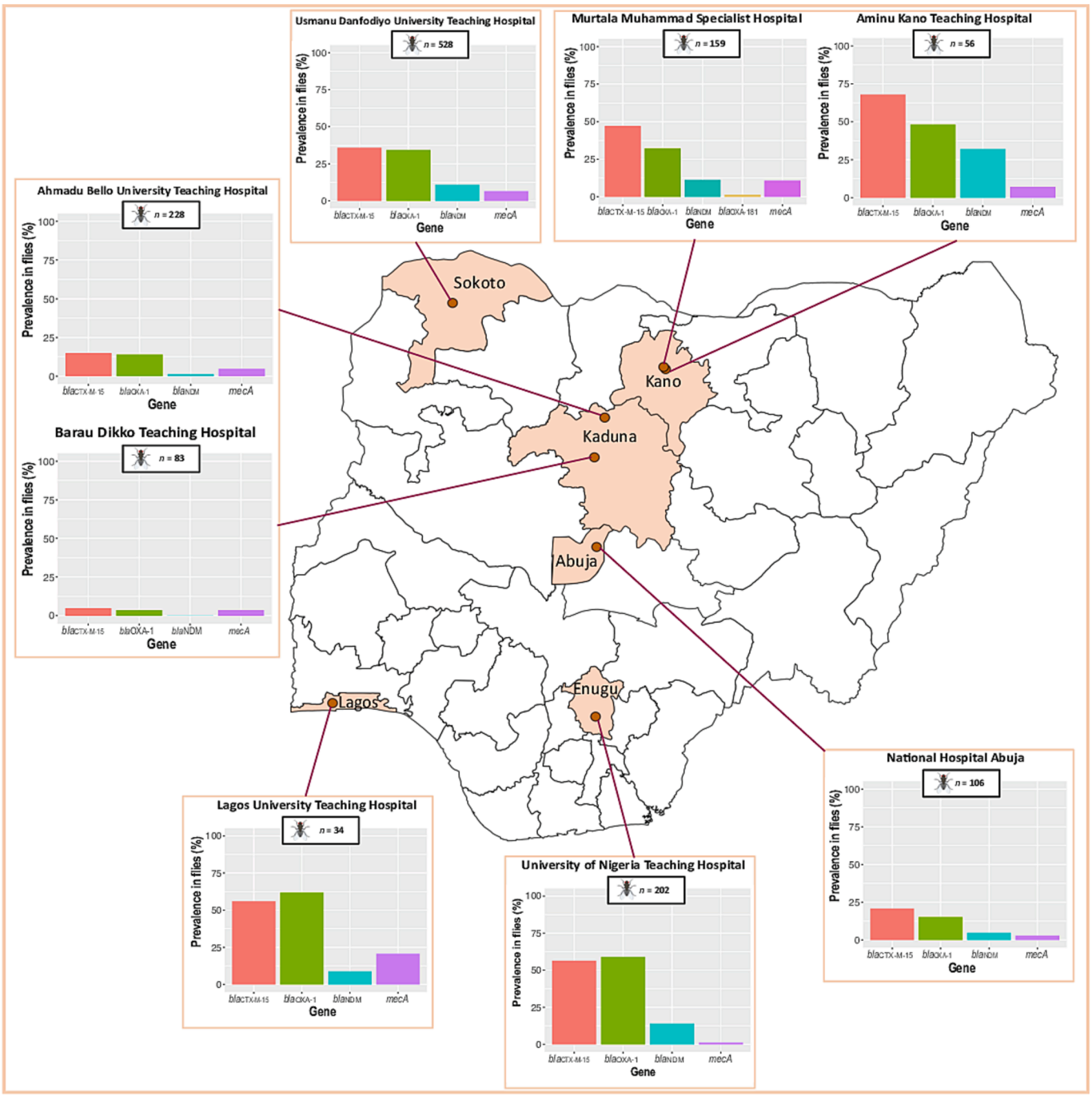

Fig. 2. Study sites where flies were collected and comparative plots for PCR prevalence of the six most ubiquitous target ARGs. Map created with the Nigeria-maps R package: https://cran.r-project.org/web/packages/naijR/vignettes/nigeria-maps.html.

was only reported in UNTH, Enugu. LUTH, Lagos had the highest percentage of GNB bacterial growth (100 %) from flies, followed by 91.6 % for UNTH, 89.3 % for AKTH, 84.9 % from MMSH, 72 % for UDUTH, 55.7 % for NHA, 43.4 % for BDTH with ABUTH having the lowest percentage of 37.7 %.

3.2. Prevalence of ARGs

ESBLs, bla_{NDM} and *mecA* genes were present at varying prevalences in bacteria isolated from flies from each hospital, except in the case of BDTH, where bla_{NDM} was not detected. ESBL genes screened were detected in bacteria from every hospital, with prevalences of bla_{OXA-1} and $bla_{CTX-M-15}$ > 25 % at five of the sites (Fig. 2). AKTH had the highest prevalence of $bla_{CTX-M-15}$ (67.9 %) and second highest prevalence of bla_{OXA-1} (48.2 %), with statistically significant pairwise differences between AKTH, UDUTH, BDTH and ABUTH in $bla_{CTX-M-15}$ prevalence (p = <0.05, Supplementary Tables 4 and 5). On the other hand, ABUTH and BDTH had the lowest prevalences of ESBLs detected, with statistically significant differences in $bla_{CTX-M-15}$ and of bla_{OXA-1} prevalence between these sites and UDUTH, MMSH, AKTH, UNTH and LUTH (p = <0.05), with the exception of no significant difference between bla_{OXA-1} prevalence between ABUTH and MMSH (Supplementary Tables 4 and 5). bla_{NDM} prevalence was > 10 % in Kano (32.1 % in AKTH, 11.3 % in MMSH), Enugu (13.9 %) and Sokoto (10.8 %). AKTH had the highest prevalence of bla_{NDM}, with significant pairwise differences between AKTH versus UDUTH, MMSH, NHA, UNTH and BDTH (p = <0.05,

Supplementary Tables 4 and 5). Conversely, both BDTH and ABUTH in Kaduna had the lowest prevalence of bla_{NDM} (0 % BDTH, 1.3 % ABUTH) with statistically significant pairwise differences seen between BDTH versus UDUTH, MMSH, AKTH, UNTH and LUTH, and between ABUTH versus UDUTH, MMSH and UNTH ($p = <0.05$, Supplementary Tables 4 and 5). The bla_{OXA-48}-like gene was present in two bacterial isolates recovered from flies from MMSH. *mecA* prevalence ranged between 7.1–20.6 %. LUTH had the greatest prevalence, with the only significant pairwise differences being between UNTH and LUTH (p = 3.07E-02, UNTH CI = [0.045, 0.089], LUTH CI [0.093, 0.384]) and UNTH and MMSH (p = 1.17E-03, UNTH CI = [0.045, 0.089], MMSH CI [0.065, 0.168]. Between hospitals sampled from the same state, no significant pairwise differences were observed, except for AKTH and MMSH (Kano) in bla_{NDM} prevalence (p = 9.30E-03, AKTH CI [0.206, 0.460], MMSH CI [0.070, 0.175]). Although there were 25–50 % growth rates on the COL and TIG plates, no samples tested positive for *tet*(X4) and *mcr-1, mcr-3, mcr-8*, *mcr-9*, or *mcr-10*.

3.3. Analysis of bacterial isolates carrying carbapenemase ARGs

132 bacterial isolates from 17 different species carrying bla_{NDM} (n = 130) or $bla_{OXA-181}$ (n = 2) were cultured from the flies (Fig. 3, Supplementary Table 6). *Providencia, Enterobacter,* and *Proteus* species were dominant bla_{NDM} carriers and bla_{NDM-1} was the most frequent gene variant (n = 79), followed by bla_{NDM-5} (n = 28), bla_{NDM-4} (n = 20) and bla_{NDM-7} (n = 3). Eight bla_{NDM} were carried on the bacterial chromosome and 124 were associated with mobile genetic elements (Supplementary Tables 7 and 8). The two $bla_{OXA-181}$-positive isolates were both *E. coli*. 79 of the isolates that tested positive for carbapenemase genes concurrently carried at least one ESBL gene (Supplementary Table 7). In total, 31 isolates belonging to *Providencia, Enterobacter, Escherichia, Klebsiella, Citrobacter* and *Morganella* species concurrently carried bla_{CTX-M} genes with $bla_{CTX-M-15}$ being most frequent (n = 29/31). The MIC distribution to meropenem ranged from ≤ 0.06 to > 128 mg/L. The majority of GNB isolates had MIC values > 32 mg/L for other β-lactam antibiotics (Fig. 4). A wide MIC distribution was also observed for gentamicin, with multiple

Fig. 3. A circus plot linking bacterial species to target ARGs and variants based on numbers of isolates that were identified as ARG-positive, as confirmed by whole genome sequencing.

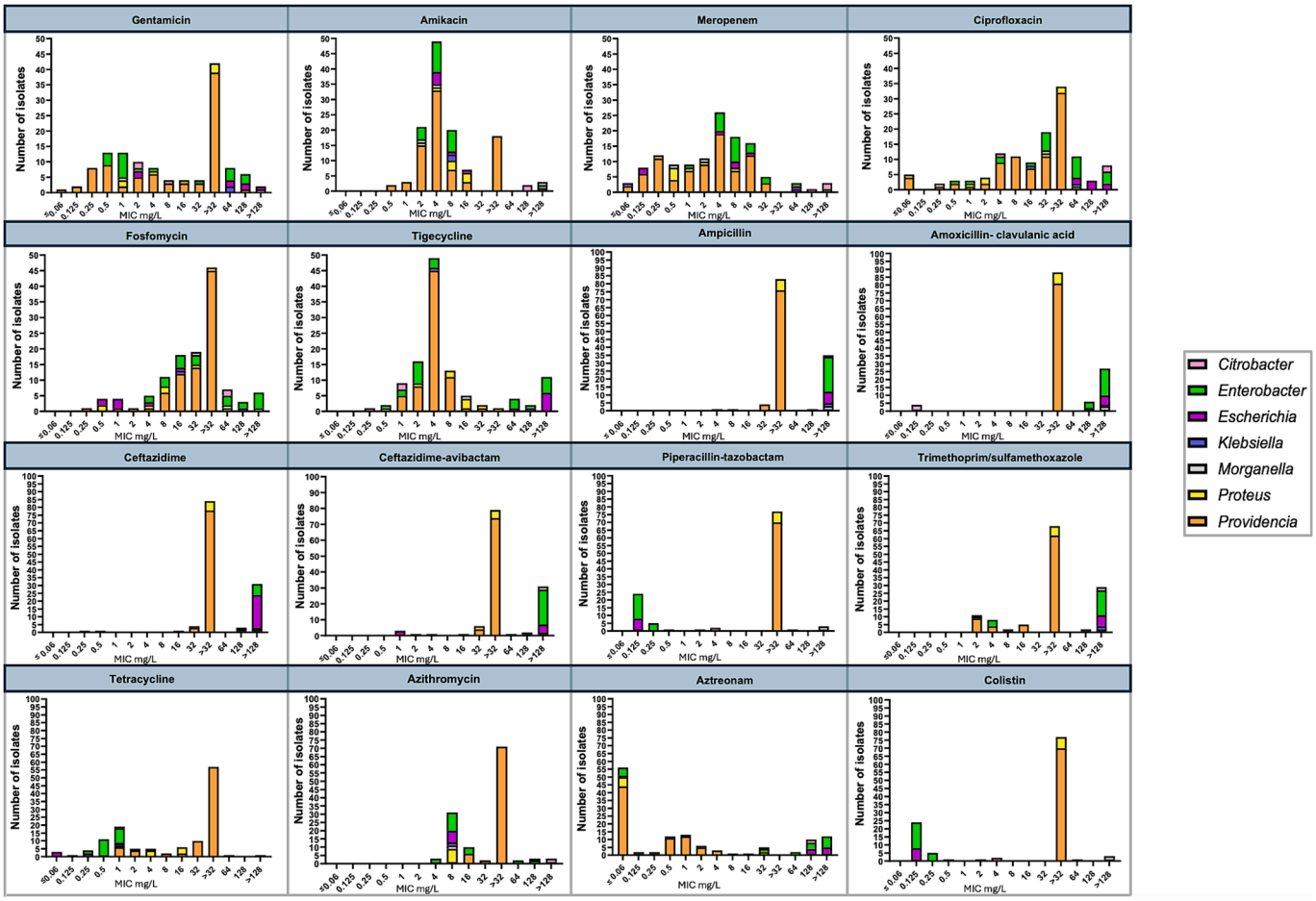

Fig. 4. Distributions of minimum inhibitory concentrations (MICs) for each antibiotic tested against the Gram-negative ARG-positive bacterial isolates (grouped and colour coded at the bacterial genus level).

aac, *ant(2″)-la* and *armA* ARGs identified (Supplementary Table 7). All isolates were considered MDR, with the highest levels of resistance observed for penicillins and cephalosporins (Fig. 4).

The most frequently identified bacterial genus with bla_{NDM} was *Providencia* (n = 83/130), with bla_{NDM-1} being the most common variant identified (Fig. 5). Most bla_{NDM} genes (n = 75/83) were harboured on plasmids across various *Providencia* species, except for eight *P. vermicola* isolates that carried bla_{NDM-1} on the chromosome. Multiple *Providencia* species had bla_{NDM-4} (n = 12/83) on IncC plasmids (Fig. 6, Supplementary Fig. 1, Supplementary Table 8). Out of the six *Providencia* species identified, the most dominant was *P. hangzhouensis,* which encompassed 49 isolates across five sequence types (STs) from five hospital sites (Fig. 5). 44/49 *P. hangzhouensis* isolates carried bla_{NDM-1} on both non-mobilisable and predicted conjugative plasmids of a variety of plasmid replicon types, as defined by rep_clusters in MOB-suite (Supplementary Table 8), whereas five *P. hangzhouensis* isolates from two different hospitals (n = 2 MMSH, n = 3 UNTH), carried bla_{NDM-4} on 10 kb – 159 kb IncC plasmids (Fig. 5, Supplementary Table 8). bla_{NDM} was also identified in *P. huaxiensis* (n = 13), *P. manganoxydans* (n = 3), *P. rettgeri* (n = 1), *P. stuartii* (n = 2), and *P. vermicola* (n = 15) (Fig. 5).

The second most frequent bla_{NDM}-positive genus was *Enterobacter,* with *Enterobacter hormaechei* (n = 21) and one *Enterobacter cloacae* cultured from five hospital sites, carrying four different bla_{NDM} variants (Supplementary Fig. 2). Most *Enterobacter* isolates had MIC values with either an increased exposure profile (4–8 mg/L) or were resistant (>8 mg/L) to meropenem and the other five β-lactams tested (Fig. 4). One bla_{NDM-1} was present on an 86.6 kb FIB/IncR plasmid with the remaining bla_{NDM-4} (n = 2), bla_{NDM-5} (n = 17) and bla_{NDM-7} (n = 2) all on predicted conjugative IncX3 plasmids (Fig. 6, Supplementary Table 8). In addition to genomic diversity amongst *Enterobacter* isolates, there were two novel STs assigned for *E. hormaechei* during this study (ST2865 and ST2866) and in total 11 different STs (Supplementary Fig. 2).

There were numerous other GNB bacteria carrying carbapenemase genes (bla_{NDM} or $bla_{OXA-181}$) including additional clinically relevant species such as *E. coli* and *K. pneumoniae.* Seven *E. coli* isolates were detected from four hospitals across multiple wards, including two carrying $bla_{OXA-181}$ carried on ~ 58 kb IncF plasmids (Supplementary Table 8). The two $bla_{OXA-181}$ positive isolates were both ST410 and recovered from different fly samples from the same hospital ward and carried $bla_{CTX-M-55}$ on different plasmids. *K. pneumoniae* was isolated in lower numbers, with three isolates cultured from flies collected from three different hospital sites including an ST307 isolate with bla_{NDM-7} on an IncX3 plasmid, and ST11 isolates carrying bla_{NDM-5}. Further bacterial diversity included *Proteus* and *Citrobacter* species. 8/11 *Proteus* spp. were *Proteus mirabilis*, five carried bla_{NDM-4} on IncC plasmids, two carried bla_{NDM-1} with limited genetic context and one carried bla_{NDM-5} on an IncX3 plasmid (Supplementary Table 8). The remaining three *Proteus* isolates were *Proteus vulgaris* carrying bla_{NDM-1} on IncT plasmids. Three *Citrobacter* species (*Citrobacter freundii*, *Citrobacter portucalensis* and *Citrobacter werkmanii*) were identified from flies circulating from three different hospitals and all had an MIC of > 128 mg/L to meropenem (Fig. 4). The *Citrobacter* spp. isolates carrying bla_{NDM-5} on IncX3 plasmids were confirmed as *C. portucalensis* and *C. werkmanii* (Fig. 6, Supplementary Table 8).

18 instances of flies co-carrying two species harbouring bla_{NDM} were identified. Of these, 14/18 involved *Providencia* spp, with 6/18 cases of

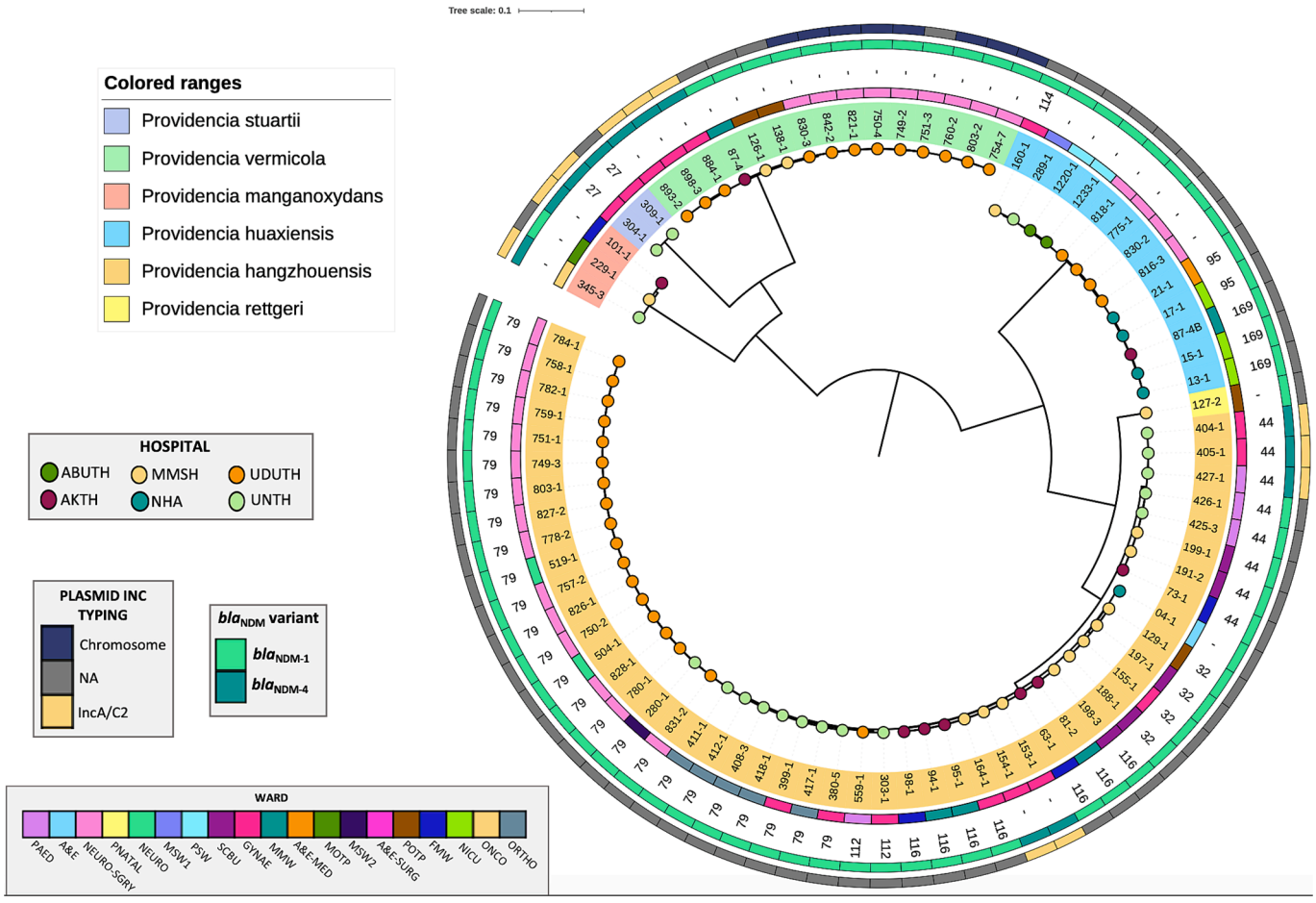

Fig. 5. A core genome phylogenetic tree of *Providencia* isolates, with tree tips colour coded according to hospital site. The inner ring immediately following the isolate name represents the hospital ward the fly sample was collected from. The sequence type (where applicable) was followed by two outer rings denoting the bla_{NDM} variant (blue-green) with the plasmid incompatibility type/chromosomal origin represented in outer ring. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

co-carriage with two different *Providencia* species, often carrying the same bla_{NDM} variant (bla_{NDM-1}), but on different plasmid types. 8/18 cases involved *Providencia* with other Enterobacterales. 3/18 cases of co-carriage involved *E. hormaechei* carrying bla_{NDM-5} on an IncX3 plasmid isolated from the same flies as *C. freundii* or *E. coli* carrying bla_{NDM-5} on an undetermined plasmid type, or *K. pneumoniae* carrying bla_{NDM-5} also on an IncX3 plasmid. One case of co-carriage involved *E. coli* carrying bla_{NDM-5} on an IncF plasmid and *K. pneumoniae* carrying bla_{NDM-5} on an undetermined plasmid type.

3.4. Analysis of bacterial isolates carrying mecA

All *mecA*-positive isolates had an MIC to cefoxitin (used as a marker of methicillin/penicillin resistance) of 8 mg/L or above (Fig. 7). Furthermore, of the 93 genomes with *mecA,* 53 concurrently carried either *aac* or *aph*, and these isolates displayed a wide range of aminoglycoside MIC values (Fig. 7, Supplementary Table 7). 86.8 % (n = 79/91 tested) of isolates carrying *mecA* were multidrug resistant. The remaining 13.2 % which were not considered MDR belonged to *Mammaliicoccus sciuri* (n = 9) and *Staphylococcus saprophyticus* (n = 3) species. The two most frequent bacterial species with *mecA* were *Staphylococcus haemolyticus* (n = 31) and *S. saprophyticus* (n = 28). Both *S. haemolyticus* and *S. saprophyticus* were recovered from six hospitals and 13 different wards in total. *S. haemolyticus* isolate 168–2 from MMSH, a novel ST (ST163) was distant in genetic relatedness to all other isolates of the same species (Fig. 8). Overall, there was high genetic variability among the *S. haemolyticus* isolates with at least 11 different STs, including four novel STs assigned herein ST160-164. There were two main branches in the *S. saprophyticus* phylogenetic tree, with many sub-clades indicating genomic diversity. There were 16 isolates of *M. sciuri* and 11/16 were from UDUTH, isolated from flies collected across four wards. *M. sciuri* was split across two main branches with two further clades in each branch including five novel *M. sciuri* STs assigned ST267-ST272. All *M. sciuri* isolates had an MIC $\geq$ 64 mg/L to cefoxitin (Fig. 7). Nine *Staphylococcus ureilyticus* isolates with *mecA* were detected from three hospitals, and six were isolates from flies from UDUTH. The remaining (n = 3) CoNS isolates were *Staphylococcus arlettae* (n = 2) with *mecA* on the chromosome, isolated from NHA and ABUTH, and a single isolate of *Staphylococcus cohnii* isolated from ABUTH.

In addition to a variety of CoNS species, six out of the 93 *mecA*-positive isolates were *S. aureus,* belonging to ST8 (n = 4), ST22 (n = 1), and ST789 (n = 1) (Fig. 8). The ST22 isolate was from UDUTH and was the only *S. aureus* isolate carrying subunits of Panton Valentine Leukocidin (LukSF-PV). Of the four ST8 isolates, two were from ATKH and two were from UDUTH and these were genetically distant depending on the hospital site (Fig. 8). Isolate 449–2 had SCC*mec* type IVa, and all other *S. aureus* isolates had SCC*mec* type V (however, this was not subtypeable, with a partial match for 254–1, 771–1 and 777–1).

4. Discussion

The threat of synanthropic flies to human health has been considered by researchers for many years; it is well documented that flies can harbour pathogenic and resistant bacteria (Rahuma et al., 2005;

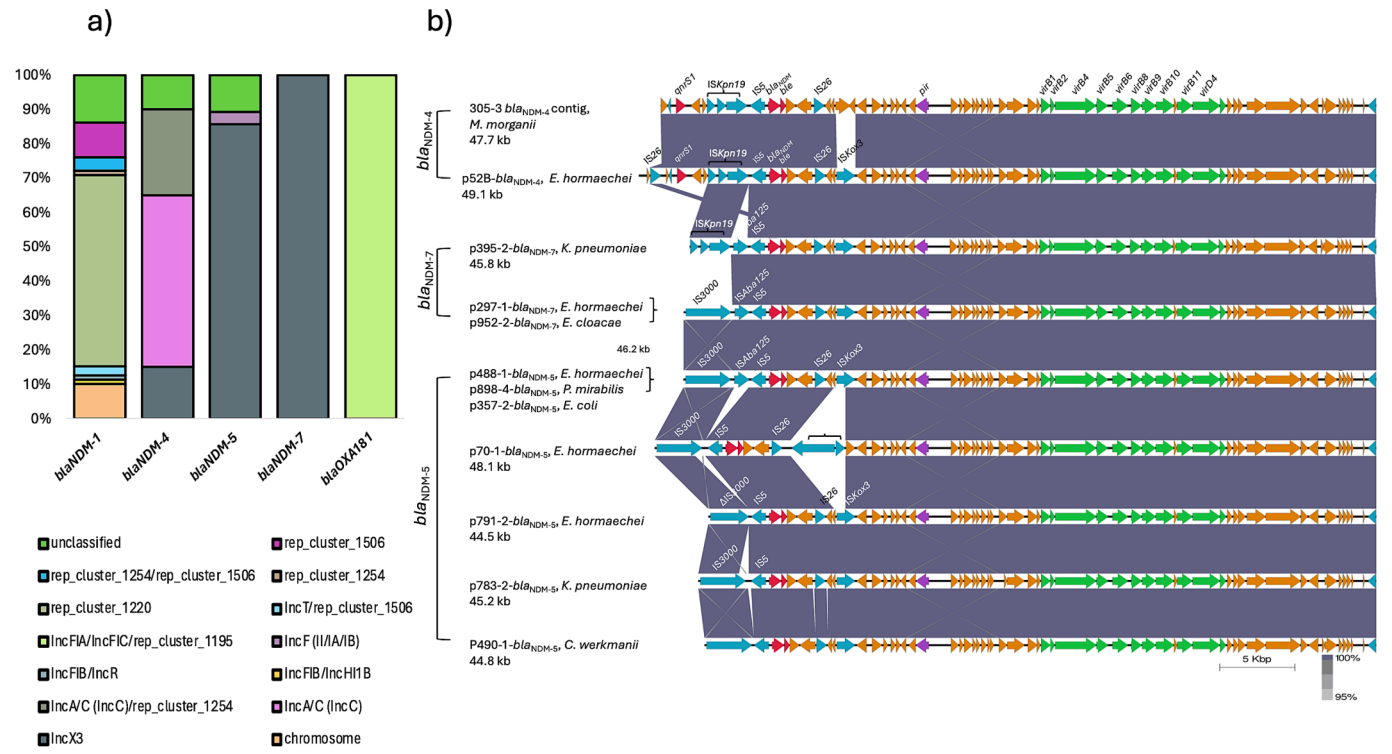

Fig. 6. A) Distribution of genomic locations for carbapenemase genes from GNB. B) Genetic maps and comparison of the IncX3 plasmids harbouring bla_{NDM-4}, bla_{NDM-5} and bla_{NDM-7} identified from different bacterial species in this study. Regions of homologous (>95 % nucleotide identity) are marked by grey shading. Arrows represent the position and transcriptional direction of genes (red: antibiotic resistance genes; light blue: mobile elements; purple: plasmid replication genes; green: plasmid conjugation genes; orange: other genes). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Onwugamba et al., 2020; Akter, 2020; Barreiro et al., 2013; Wetzker, 2019; Ommi et al., 2015). Previous hospital-based studies have either assessed a single site, worked on a low number (1–100) of total fly samples or focused on certain bacterial species or resistance genes (Heiden, 2020; Fotedar et al., 1992; Wiktorczyk-Kapischke, 2022), making it difficult to generalise the results. This multi-site surveillance study demonstrated that the carriage of AMR bacteria by flies is a potential problem throughout Nigeria, with varying levels of ESBL, carbapenemase and *mecA* resistance genes detected in all regions assessed.

The microbiome of the fly is largely affected by the environment in which it lives and stochastic events (Park, 2019; Junqueira, 2017), though there are certain bacterial species that are particularly abundant and commonly identified in the microbiota, including *Providencia* spp. (Junqueira, 2017; Zurek et al., 2000). The average prevalence of ESBL-positive Enterobacterales observed in this study is significantly greater than the rate of 0.8 % which has been reported from flies in non-clinical environments in Nigeria (Onwugamba et al., 2020). A comparable hospital setting in Ethiopia observed an 80 % prevalence of ESBL-positive GNB in flies (Tufa, 2020). A review of data regarding hospitalised patient's infections across Nigeria found prevalences of ESBL carriage ranged between 7.5 % and 82.3 % (Tanko et al., 2020). This may suggest that that the ARGs detected in flies in hospital settings are associated with the hospital setting itself, which could be related to the increased density of ARGs caused by antimicrobial use in the clinical environment (Hassoun-Kheir, 2020).

Carbapenem-resistant Enterobacterales (CRE) remain critical pathogenic threats according to WHO (WHO bacterial priority pathogens list, 2024). Investigating flies as transmission vectors of CRE raises questions about the directionality and dynamics of bacterial and plasmid dissemination. Considering that *Providencia* spp. were isolated ubiquitously in flies across sites, accounted for the greatest number of bla_{NDM}-positive isolates and constituted the most diverse bla_{NDM}-positive genus – with the majority of bla_{NDM} carried on diverse plasmids - it may be possible that *Providencia* spp. within the fly's microbiome can transfer bla_{NDM} to other Enterobacterales in LMIC hospitals. Gan *et al.,* recently studied plasmid dissemination in houseflies in China and identified *Providencia* as the recipient of an *mcr-8* containing plasmid, with conjugation assays confirming that this could be readily transferred to clinically relevant isolates of *Klebsiella pneumoniae* (Gan, 2024). Acquisition of bla_{NDM} by *Providencia* spp. is a clinically concerning finding as members of this genus are intrinsically resistant to commonly utilised antibiotics including some aminoglycosides, penicillins, first and second generation cephalosporins, as well as reserve antibiotics such as colistin, leaving carbapenems as one of the few antibiotic classes available for treatment of serious *Providencia* spp. infections (Magiorakos, 2012; O'Hara et al., 2000; Rajni, 2022). This is exemplified by *Providencia* spp isolated in this study exhibiting MDR phenotypes. The *Providencia* genus has recently undergone a reclassification and expansion, as novel species are frequently being identified and characterised by WGS analysis (Dong et al., 2024). In the present study, bla_{NDM-1} was associated with a range of *Providencia* species. The overlap between human- and animal-associated *Providencia* lineages and the increasing reports of MDR *Providencia* clinical infections warrant further investigation into the AMR threat of *Providencia* harboured by flies in clinical settings.

Enterobacter and *Klebsiella* are also CRE that cause carbapenem-resistant infections in Nigeria (Sands, 2021) and have been detected on hospital surfaces in NHA and MMSH (Nieto-Rosado, 2024). Evidence for likely dissemination networks of CRE such as *E. cloacae, E. coli* and *K. pneumoniae* between flies and hospital surfaces have been identified in Pakistan (Hassan *et al,* 2021), which is a phenomenon that should also be investigated for sites in Nigeria as flies may act as direct or indirect distributors of CRE to patients by acting as living, moving vehicles for the distribution of bacteria around hospital surfaces, as well as by

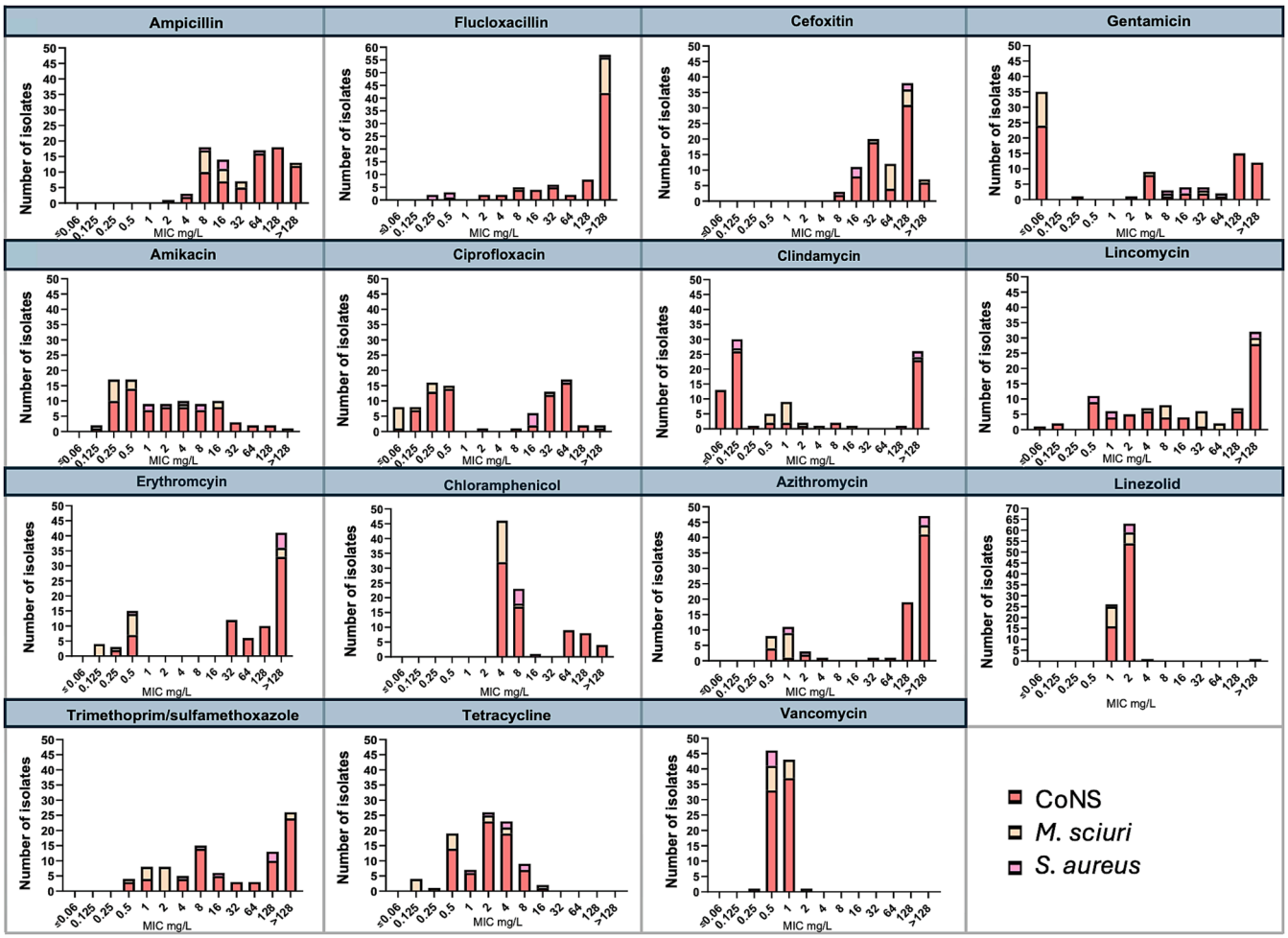

Fig. 7. Distributions of minimum inhibitory concentrations (MICs) for each antibiotic tested against the Gram-positive mecA-positive bacterial isolates (grouped and colour coded as Coagulase-negative Staphylococci (CoNS), *M. sciuri* and *S. aureus*)).

landing directly on patients' bodies. The fact that no bla_{NDM} was detected in BDTH may be a reflection of the small sample size ($n = 83$), though there was also only a 1.3 % prevalence in ABUTH ($n = 228$), so these finding may in fact corroborate previous investigations that did not detect bla_{NDM} within hospitals in Kaduna (Suwaiba et al., 2020), which is surprising considering the significantly higher bla_{NDM} prevalence rates in neighbouring states, such as Kano in this study.

Methicillin resistant *S. aureus* (MRSA) also remains a high priority for WHO (WHO bacterial priority pathogens list, 2024), and though only isolated from few flies (n = 6) these isolates all tested positive for *mecA* and the majority were ST8, which often causes human infections (Ibrahim, 2023; Sands, 2022; Schaumburg et al., 2014). The sampling for this pilot study took place towards the end of the rainy season in Nigeria where temperatures were relatively low compared to in the summer, during which the prevalence of MRSA may have been higher (Sobur, 2022). The large intraspecies diversity within the *mecA*-positive CoNS isolates suggests the presence of a diverse *Staphylococci* community within the fly microbiome, which is concordant with existing studies (Park, 2019; Abdolmaleki et al., 2019; Bahrndorff et al., 2017; Sudagidan, 2022). *S. saprophyticus* is a common cause of urinary tract infection (UTI) in sexually active young women and has also been recovered from the gastrointestinal tract of animals and the broader environment (Lawal, et al., 2021). In this study, 28 *S. saprophyticus* isolates were identified from multiple flies across hospital sites in Nigeria, including six flies caught in a gynaecology ward in MMSH, Kano. High levels of MDR were exhibited by *mecA*-positive isolates from flies, including those of the *M. sciuri* species.

The isolation of several ARG-carrying bacteria from flies such as *Providencia* spp. and CoNS, which are commonly found in animal and environmental sampling and are known to cause infections such as UTI's, catheter-associated infections and surgical site infections calls for further exploration of the role of flies in the dissemination of such opportunistic pathogens between environmental, animal and human settings (Schaumburg, 2016). Although previous genomic analysis has highlighted significant genetic similarities in bacteria isolated from flies, hospital surfaces and patients (Hassan et al, 2021), further investigation is required to understand to what extent and by which mechanisms insect pests contribute towards source-sink transmission dynamics of ARGs in clinical environments.

The main objective of this pilot study was to develop a high throughput method for the surveillance AMR bacteria carried by flies in hospitals. Several methodical improvements will be implemented in the subsequent multinational study to address limitations garnered during this study. Firstly, we plan to use Amies Transport Medium to limit compositional microbiota changes due to uncontrollable variations in sample transit times, which may have impacted the recovery of the fly microbiota to varying degrees between the sites sampled, influencing the ARG prevalence and diversity of bacteria isolated. Furthermore, a selective agar approach was utilised, which enabled the culture of swarming bacteria including *Providencia* and *Proteus* species but may have led to under-representation in the growth of non-swarming species (a common phenomenon when swarming species are present in a mixed sample (Smith, 1972). An additional broth culture step for more representative recovery of bacterial species diversity will be conducted

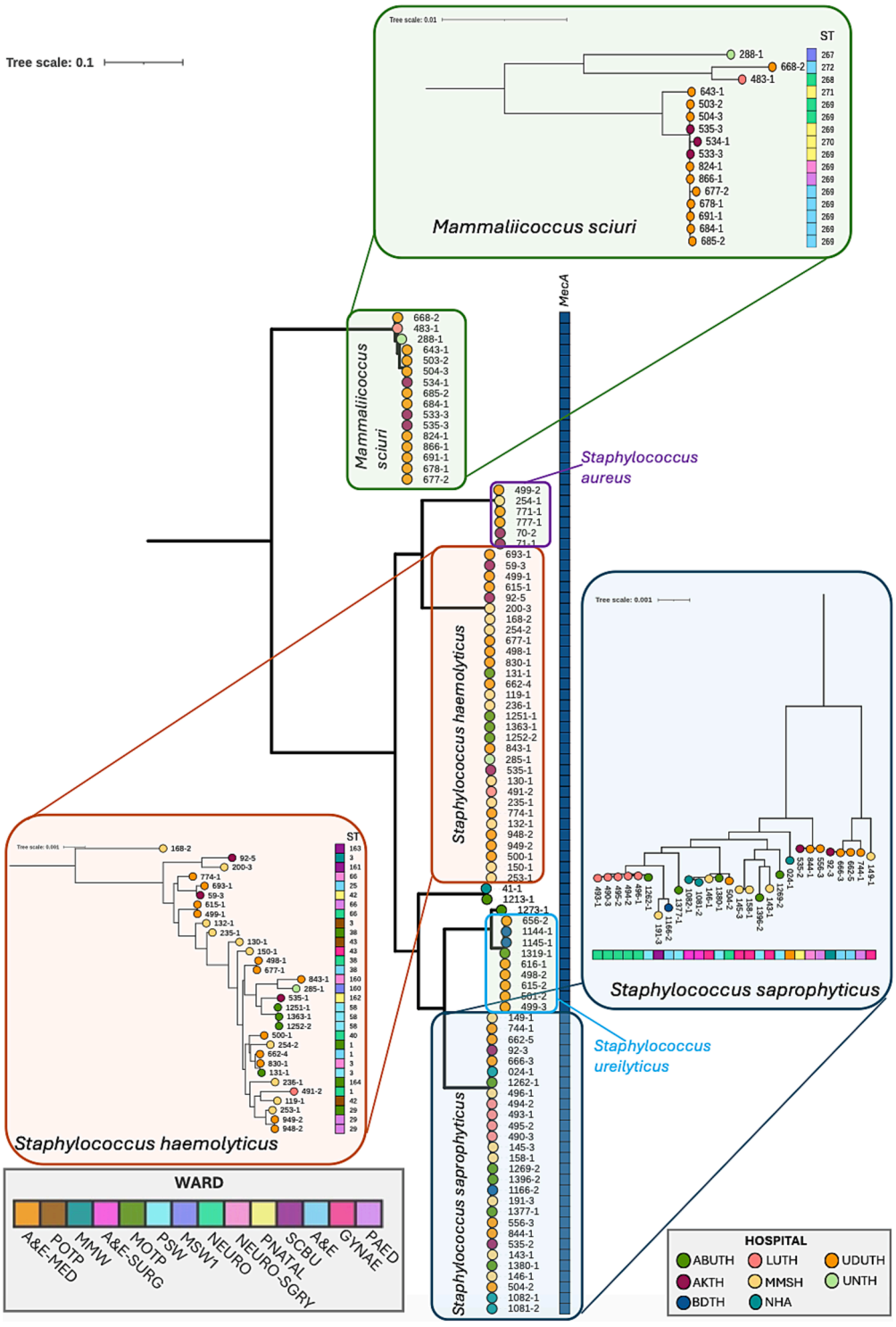

Fig. 8. A core genome phylogenetic tree of *Staphylococci* species with composite smaller phylogenies generated for each coagulase negative species to improve resolution and comparison. The strip immediately following the isolate name represents the hospital ward the fly sample was collected from followed by sequence type. The outer strip on the large phylogeny denoted the presence of the *mecA* gene.

alongside the existing method of agar culture conducted in this workflow. Flies sampled during the multinational study will also be subjected to metagenomic analysis to uncover the total resistome harboured by flies in the absence of culture-bias or resistance marker selection limits. This will be particularly useful for the analysis of sites whereby although there is bacterial growth, target ARGs are not detected and so other resistance mechanisms may be at play, which may account for the low prevalence of bla_{NDM} in Kaduna in this study. Additionally, molecular speciation of flies will be conducted in order to speciate flies in a less subjective manner to aid further understanding of the role of specific fly species in the carriage of AMR bacteria, which was a significant limitation in this study.

The abundance of ARGs in flies across Nigerian hospitals is an alarming insight into the potential threat of AMR in these settings. The vast abundance of flies circulating within these hospitals, as demonstrated by the collection of over 1000 flies within a short time frame, is

in part due to favourable climates enabling an overabundance of flies. Climate change is expected to alter the climate of temperate countries to those more tropic and permissive in sustaining an overpopulation of flies. Factors such as uneven sampling, single sampling events (not accounting for seasonality), and sampling sites all being within a single country limited our ability to detect any associations between climatic variables and ARG prevalence in this study, but it is already known that higher seasonal temperatures are associated with an increase in *mecA*-positive *S. aureus* detected in houseflies (Sobur, 2022). Data gathered through multi-site surveillance on an international scale will allow modelling estimations to be made on the potential role of flies in the propagation of AMR in response to climate change predictions. In tandem with this, the retrieval of clinical data surrounding antibiotic usage and IPC considerations will be used to determine the influence of antibiotic usage and pest control measures on the prevalence of AMR in flies circulating within hospitals internationally.

The AVIAR multinational study intends to further survey the role of flies as sentinels of AMR in hospitals across the globe, in response to recent calls for the need for *"active surveillance of the quantity and quality of Antimicrobial Resistance appearing in insects"* (Rawat, 2023).

5. Conclusion

Flies circulating within hospitals throughout Nigeria carry a diverse community of multidrug-resistant, clinically significant bacterial species. These findings validate the ability of flies to harbour AMR bacteria in clinical settings and highlight the need for further understanding the role of flies as vectors of AMR and infection. This pilot study has illuminated areas where method development will enhance the subsequent multinational AVIAR study.

Author contributions

CA, TRW, and KS designed the study. SPB, KC, CA and KS prepared the manuscript, with contributions from all authors. CA and COR coordinated the shipment of flies and study logistics. CA, SBP, KC, MNR and EARP performed the microbiological processing of flies. KC, JM, JA and KS performed the whole genome sequencing. KS, KC, and ML performed bioinformatics analysis. KI, SLA, AA, YM, IN, OO, ZT and AI were the study site leads facilitating the collection of flies and data from each hospital.

7. Author statement

All listed authors contributed to the manuscript as described in the Author contributions section (below). All authors have given full permission to the corresponding author to proceed with publication of the manuscript, and the corresponding author takes full responsibility for associated contact requests.

CRediT authorship contribution statement

Kate Cook: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Shonnette Premchand-Branker:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Maria Nieto-Rosado:** Methodology, Investigation, Formal analysis, Data curation. **Edward A.R. Portal:** Methodology, Data curation. **Mei Li:** Visualization, Investigation, Formal analysis. **Claudia Orbegozo Rubio:** Project administration. **Jordan Mathias:** Data curation. **Jawaria Aziz:** Data curation. **Kenneth Iregbu:** Writing – review & editing, Project administration. **Seniyat Larai Afegbua:** Writing – review & editing, Project administration. **Aminu Aliyu:** Project administration, Data curation. **Yahaya Mohammed:** Project administration, Data curation. **Ifeyinwa Nwafia:** Project administration, Data curation. **Oyinlola Oduyebo:** Project administration, Data curation. **Abdulrasul Ibrahim:** Project administration, Data curation. **Zainab Tanko:** Project administration, Data curation. **Timothy R. Walsh:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Chioma Achi:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Project administration, Supervision. **Kirsty Sands:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Project administration, Supervision.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2025.109294.

Data availability

All genomes are available within the NCBI repository under the project accession number PRJNA1141257.

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