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Hospital-Associated Flies as Potential Reservoirs of Antimicrobial-Resistant Bacteria: An Entomological and Molecular Investigation in Iraq

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ABSTRACT:

Background: In Iraq, it has not been characterized how the synanthropic flies in the hospital ward and the waste disposal and the surrounding community can transmit an ARB. This study used a combination of entomological, microbiological and molecular approaches to investigate the carriage of clinically important ARB by flies in hospitals in Baghdad.

Methods: Adult flies (n=432) were collected between March and October 2024 from ICUs, surgical wards, hospital kitchens and waste disposal locations of three tertiary hospitals, and from community control sites located at least 1 km away. The cultures of external body surfaces and gut homogenates were maintained separately. The susceptibility testing of isolates was done by CLSI M100 (34th edition) as per conventional and automated methods and these were screened for ESBL, carbapenemase, colistin and other resistant genes by PCR.

Results: Most of the flies captured were Musca domestica (70.6%). In total, 384 isolates were recovered as a result of 91.2% of flies having at least one bacteria with a clinical background. More than fifty percent of Escherichia coli and Klebsiella pneumoniae were resistant to third-generation cephalosporins; Acinetobacter baumannii had carbapenem resistance of 67.7%. Two-thirds of resistance were observed in Escherichia coli with most of them constitutively AmpC strain with the methylation of the tetM gene only occurring in 1 strain. The gene blaCTX-M-1 was identified in 52.4% of Enterobacterales and blaNDM-1 in 15.3% of K. 44.7% Staphylococcus aureus has a mecA.

Conclusions: Flies from hospitals in Baghdad carry a substantial burden of multi-drug resistant organisms which include WHO critical-priority pathogens. In addition, they constitute mobile reservoirs which are capable of linking clinical, environmental and community resistomes. In Iraq infection prevention and antimicrobial stewardship programmes must integrate fly surveillance and control.

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1. INTRODUCTION

One of the top ten global public health threats is antimicrobial resistance (AMR). In 2019, 1.27 million deaths were directly attributed to bacterial AMR, while nearly five million more were associated with that condition, a burden that is born disproportionately by low- and middle-income countries (Antimicrobial Resistance Collaborators, 2022). The World Health Organization has categorized enterobacterale that are resistant to third-generation cephalosporins and carbapenems as well as carbapenem-resistant Acinetobacter baumannii and carbapenem-resistant Pseudomonas aeruginosa as critical priority pathogens in urgent need of novel therapeutic options. Iraq is a case in point: the first national survey of its surveillance network recorded that 80% of A. Certain strains of A. baumannii are showing high resistance to cephalosporin antibiotics. Both the Baumannii and E species. Escherichia coli and a methicillin resistant Staphylococcus aureus (MRSA) prevalence (74%) (Alhilfi et al., 2023). Molecular studies conducted on a clinical isolate from Iraqi hospitals have confirmed the circulation of international high-risk clones carrying blaNDM-1, blaOXA-23 and blaCTX-M-15 (Ganjo et al., 2016; Hussein, 2018; Ismail & Mahmoud, 2018; Kareem et al., 2025). Decades of conflict, fragmented surveillance and widespread empirical antibiotic use have aggravated the situation (Abou Fayad et al., 2023; Al-Jumaili & Ahmed, 2024).

Efforts to control AMR in hospitals have focused on hand hygiene, patient isolation, environmental cleaning and antimicrobial stewardship. Addressing transmission through personnel and fomites should involve more about mobile biological vectors. Flies that adapted to human lifestyle, especially house fly and blow flies, have morphometric and behavioural features that make them efficient mechanical vectors for infectious disease agents. They possess sponging mouthparts, hairy bodies, and tarsal pulvilli adapted for trapping micro-organisms. Flies are known to feed on excreta including urine and faeces, refuse, and wound exudates (blood and pus). Their flight range may extend over several kilometers – most notably between hospitals, markets, and households.

A systematic review recorded over 130 human pathogens associated with the house fly (Khamesipour et al., 2018), and the gut of M. The microbiota found in domestica includes more environmental origin strains as opposed to maggot, which is essential for allowing horizontal exchange of resistance determinants within the dominant Proteobacteria and Firmicutes (Bahrndorff et al., 2017; de Jonge et al., 2020; Fukuda et al., 2016).

There has been a rapid accumulation of evidence that flies carry resistant bacteria in and out of hospitals. In Ethiopia, flies trapped inside a referral hospital compound had 67% ESBL-producing Gram-negative bacteria, in comparison to only 2% of flies trapped from a butchery 1.5 km away (Tufa et al., 2020). A tertiary hospital located in Rwanda had flies that have MDR pathogens. Escherichia coli strain with pandemic sequence type 131 (Heiden et al., 2020). In Nigeria, resistance genes, including ESBL genes, blaNDM and mecA were found in flies in samples from eight hospitals (Achi et al., 2025). In Germany, breeding of moth flies in hospital wastewater biofilms was implicated in the spread of MDR Stenotrophomonas maltophilia (Rupprecht et al., 2020). In separate works done in places like Iran, Libya, Thailand and South Africa researchers have isolated MDR enteric bacteria and staphylococci from hospital flies repeatedly (Fotedar et al., 1992; Monyama et al., 2023; Nazari et al., 2017; Punyadi et al., 2021; Rahuma et al., 2005; Ranjbar et al., 2016).

Over the years, several authors have proposed that flies can be potential vectors and environmental sentinels of AMR (Gwenzi et al., 2021; Onwugamba et al., 2018).

With the AMR crisis in Iraq being very severe and the presence of high densities of synanthropic flies in Iranian hospitals, no study is yet conducted to investigate the bacterial burden, resistance phenotypes and resistance gene content of hospital-associated flies in Iraq. This gap is important. If flies shuttle resistant organisms between patients, hospital waste, and the kitchen and surrounding community, then control strategies that do not include them will remain incomplete. The aim of present study was to characterize composition of fly species from three tertiary hospitals in Baghdad; to isolate and identfy clinically important bacteria from their external surface and gut; to evaluate antimicrobial susceptibility pattern and MDR status of isolates; and detection of the key resistance genes ESBL, carbapenemase, mecA, vanA/vanB and mcr-1 by polymerase chain reaction (PCR.).

2. MATERIALS AND METHODS

2.1 Study design, setting and period

Between March and October 2024, a cross-sectional entomological and microbiological study was carried out between in Baghdad, Iraq in three public tertiary hospitals (designated Hospitals A, B and C, with 900–1,200 beds each). The four functional areas sampled in each hospital were intensive care units (ICU), surgical wards, hospital kitchens and outdoor waste disposal areas. The community control sites, which were residential markets and food-handling premises located at least 1 km away from each hospital and not connected to their drainage, provided a comparative baseline by being sampled in parallel.

2.2 Fly collection and entomological identification

Field adult monitoring was carried out starting from July 1998 in a Limited Area of Kenya. Adult flies were collected through the use of sterile sweep nets in indoor areas and non-toxic baited traps in kitchens, waste areas and community sites between 09:00 and 13:00, which was the time of peak flight activity. They were collected twice monthly. Traps were placed for no more than 2 h to limit desiccation and post-capture contamination. Every sample placed in separate sterile container after aseptic addition and transported to lab in a carton with ice pack within 2 h of catch. Due to determination below species level, specimens that were too damaged or deformed to allow reliable identification were not included.

2.3 Specimen processing and bacterial isolation

To differentiate between external carriage and internal carriage, each fly was processed in two fractions. The entire fly was agitated for a minute in 2 mL of sterile 0.85% saline to obtain the external fraction. The fly was then surface sterilized by treating in 70% ethanol (for 1 min) followed by two rinses in sterile saline. Subsequently, the fly was macerated in 2 mL of saline to obtain the gut fraction. 100 microliters of each suspension, as well as 10-fold serial dilutions, were spread onto blood agar, MacConkey agar, mannitol salt agar and cetrimide agar and incubated aerobically at 37 °C for 18–24 h. Pure forms were achieved by sub-culturing. Estimation of viable counts was done using dilution plates having 30-300 colonies.

2.4 Identification and antimicrobial susceptibility testing

The isolates were identified using Gram staining, observation of colony morphology and a standard biochemical panel. This includes oxidase, catalase, coagulase, urease, triple sugar iron, indole and citrate reactions. Moreover, confirmation of the identification is performed for a randomly selected 20% subset. This was done by 16S rRNA gene amplification and Sanger sequencing. The Kirby-Bauer disk diffusion method was used for antimicrobial vulnerability testing (AST) on8 Mueller-Hinton agar and results were interpreted according to Clinical and Laboratory Standards Institute M100, 34th edition (2024). The panel consisted of beta-lactams (ampicillin, amoxicillin-clavulanate, etc.), cephalosporins (cefotaxime, ceftazidime, etc.), carbapenems (imipenem, meropenem), fluoroquinolones and aminoglycosides for Gram-negative bacilli; beta-lactams (penicillin, cefoxitin, etc.) and vancomycin, linezolid for staphylococci; vancomycin, linezolid, high-level gentamicin for enterococci. The MICs of Colistin for Gram-negative isolates was determined by broth microdilution. S. suberifaciens, E. coli, Pseudomonas and Escherichia coli for Testing. The strain ATCC 25923 of aureus was used to confirm the quality of strains.

2.5 Phenotypic detection of ESBL and carbapenemase production

Ceftazidime and cefotaxime disk test was used to screen Enterobacterales for ESBL production which was confirmed by double disk synergy test with amoxicillin–clavulanate. The modified carbapenem inactivation method with EDTA-modified CIM was used to assess carbapenemase production in carbapenem non-susceptible isolates. S. Methicillin resistance in The cefoxitin disk results (≤21 mm) suggested that aureus and mecA PCR confirmed it.

2.6 DNA extraction and molecular detection of resistance genes

Genomic DNA from pure cultures was extracted by boiling-lysis method and stored at −20 °C. The PCR procedure was conducted for a number of targets including blaTEM, blaSHV and blaCTX-M (groups 1, 2 and 9), blaNDM, blaVIM, blaKPC, blaOXA-48-like and blaOXA-23-like as well as mecA, vanA and vanB, mcr-1. A 1.5% agarose gel containing a safe stain for nucleic acids was used to resolve the amplicons. Visualization was achieved under ultraviolet transillumination with a

100 bp ladder. The amplicons of representative positive isolates were subjected to Sanger-sequencing for confirmation and sequences were queried against GenBank using BLASTn.

2.7 Definitions

In accordance with international consensus definitions, isolates were classified as multidrug-resistant (MDR), extensively drug-resistant (XDR) or pandrug-resistant. A fly was labelled as an MDR carrier when at least one MDR organism was recovered from its either of its fractions.

2.8 Statistical analysis

The information analyzed in IBM SPSS Statistics version 27. Proportional computed by chi-square test or Fisher's exact test as appropriate and odds ratios (ORs),95% confidence intervals (CIs) hospital-vs-community comparisons. A two-sided p value < 0.05 was regarded as statistically significant.

2.9 Ethical considerations

The ethics committee of the university that participated in the study reviewed the protocol and gave approval. The hospital administration also gave their written consent. No samples or patient-identifiable data were collected for patients. Entomological sampling in clinical areas was scheduled so as not to interfere with patients.

3. RESULTS

3.1 Entomological findings

A sum of 432 adult flies was collected of which 318 (73.6%) were from hospital sites and 114 (26.4%) were from community control sites (Table 1). The predominant species at all 432 sites was Musca domestica (305/432; 70.6%), followed by Chrysomya megacephala (61/432; 14.1%), Lucilia sericata (39/432; 9.0%) and Sarcophaga spp. Six point three percent The M's comparable abundance. The relative abundance of domestica was greatest in kitchens (55/72; 76.4%) and community sites (78/114; 68.4%), while blow flies and flesh flies were proportionately more abundant in ICUs and waste disposal sites.

Table 1. Species composition of flies collected at hospital and community sites, Baghdad, March–October 2024.

Sampling site	*M. domestica*	*C. megacephala*	*L. sericata*	*Sarcophaga* spp.	Total
Intensive care units	66	12	10	8	96
Surgical wards	58	10	9	7	84
Hospital kitchens	55	9	4	4	72
Waste disposal areas	48	11	4	3	66
Community sites	78	19	12	5	114
Total (%)	**305 (70.6)**	**61 (14.1)**	**39 (9.0)**	**27 (6.3)**	**432 (100)**

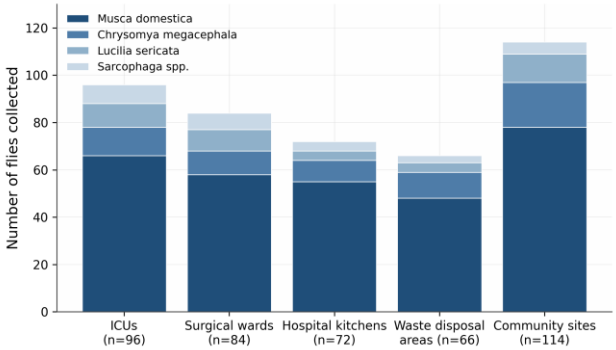

Figure 1. Species composition and distribution of the 432 flies collected across hospital and community sampling sites.

3.2 Bacterial carriage and isolate distribution

From the collection of 432 flies, 394 (91.2%) recovered one or more bacterial species of clinical significance. The hospital flies were more often carrying the resistant bacteria than a community fly (95% v/s 80% - that is more likely 20 times more likely) In total, 384 non-duplicate isolates were obtained of which the external body washes had 216 (56.3%) and gut homogenate had 168 (43.7%), in all (Table 2) Gram-negative bacilli predominated (283 / 384; 73.7%), with E. K. estimated (96/384;25.0%) of E. coli. pneumonia (72/384; 18.8%) Acinetobacter baumannii (31 isolates) was mainly obtained from hospital flies (27/31; 87.1%) with 21 of the 31 isolates (67.7 %) from ICUs or surgical wards. Quantitative cultures of external washes yielded counts from 2.1×10^3 to 3.4×10^6CFU/ fly (median 6.8×10^4CFU), with maximum load being from flies captured from waste dump sites.

Table 2. Distribution of bacterial isolates by species, anatomical fraction and sampling origin (n = 384).

Species	External surface	Gut	Hospital	Community	Total (%)
Escherichia coli	55	41	68	28	96 (25.0)
Klebsiella pneumoniae	38	34	55	17	72 (18.8)
Pseudomonas aeruginosa	24	20	36	8	44 (11.5)
Staphylococcus aureus	24	14	26	12	38 (9.9)
Enterococcus faecalis	19	16	20	15	35 (9.1)
Acinetobacter baumannii	19	12	27	4	31 (8.1)
Enterococcus faecium	15	13	18	10	28 (7.3)
Enterobacter cloacae complex	12	10	16	6	22 (5.7)
Proteus mirabilis	10	8	11	7	18 (4.7)
Total	**216**	**168**	**277**	**107**	**384 (100)**

3.3 Antimicrobial susceptibility

The resistance rates of Gram negatives to almost all the drug classes were high (Table 3, Figure 2). Resistance to third-generation cephalosporins was detected in E. coli Cefotaxime resistance in 65.3% of Ks. pneumoniae. The resistance of A to carbapenem reached 67.7%. 36.4% in P. aueruginosa (meropenem), 22.2% in K. 8.3% of E pneumoniae in E.coli. Colistin remained mostly effective, with resistance in 9.7% of A. 2.3% of P. aeruginosa; however, two E. baumannii and two K and E coli isolates of pneumoniae were resistant colistin. 44.7% of S. in Gram-positive cocci. (Table 4) 25% of E. faecalis strains were resistant to vancomycin, while all MRSA strains exhibited resistance to methicillin. faecium and 8.6% E. VanA was carried by all E. faecalis strains, whereas one E. faecium strain carried vanB. No isolation of S. The aureus species was impervious to vancomycin or linezolid.

Table 3. Antimicrobial resistance rates (%) among Gram-negative bacilli isolated from flies (NT, not tested/intrinsically resistant).

Agent	*E. coli* (n = 96)	*K. pneumoniae* (n = 72)	*P. aeruginosa* (n = 44)	*A. baumannii* (n = 31)	*E. cloacae* (n = 22)	*P. mirabilis* (n = 18)
Ampicillin	89.6	97.2[a]	NT	NT	NT	72.2
Piperacillin–tazobactam	27.1	45.8	43.2	74.2	31.8	NT
Cefotaxime	58.3	65.3	NT	NT	50.0	38.9

Agent	*E. coli* (n = 96)	*K. pneumoniae* (n = 72)	*P. aeruginosa* (n = 44)	*A. baumannii* (n = 31)	*E. cloacae* (n = 22)	*P. mirabilis* (n = 18)
Ceftazidime	54.2	62.5	56.8	80.6	45.5	35.0
Cefepime	51.0	58.3	50.0	77.4	36.4	33.3
Ciprofloxacin	47.9	51.4	52.3	77.4	40.9	33.3
Gentamicin	38.5	44.4	47.7	74.2	36.4	27.8
Meropenem	8.3	22.2	36.4	67.7	13.6	5.6
Trimethoprim–sulfamethoxazole	66.7	58.3	NT	71.0	45.5	61.1
Colistin[b]	2.1	2.8	2.3	9.7	0	0

[a] Intrinsic resistance expected. [b] Determined by broth microdilution.

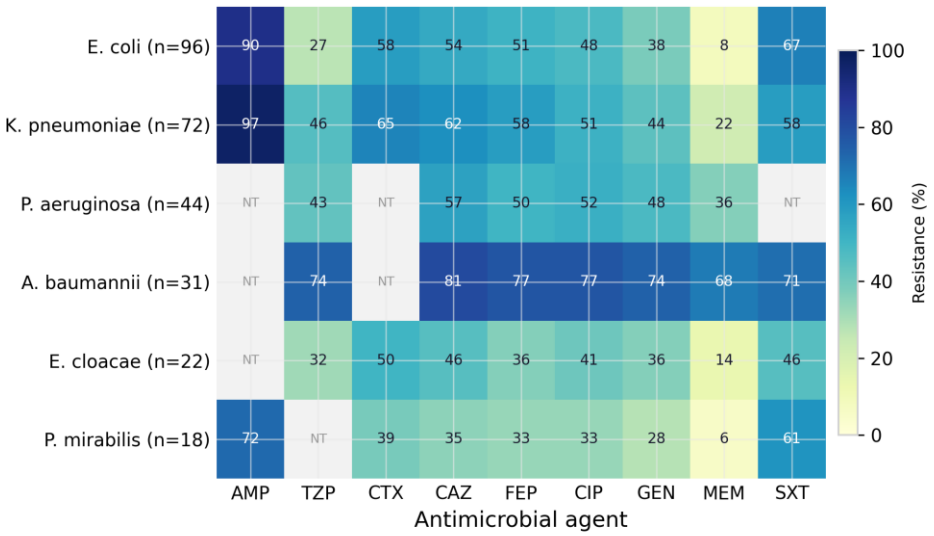

Figure 2. Heat map of antimicrobial resistance rates among Gram-negative bacterial isolates recovered from hospital and community flies. Values denote the percentage of resistant isolates; NT, not tested or intrinsic resistance.

Table 4. Antimicrobial resistance rates (%) among Gram-positive cocci isolated from flies.

Agent	*S. aureus* (n = 38)	*E. faecalis* (n = 35)	*E. faecium* (n = 28)
Penicillin/ampicillin	92.1	22.9	85.7
Cefoxitin (MRSA screen)	44.7	—	—
Erythromycin	52.6	60.0	71.4
Clindamycin	39.5	—	—
Gentamicin / high-level gentamicin	31.6	45.7	57.1
Ciprofloxacin	42.1	51.4	67.9

Agent	*S. aureus* (n = 38)	*E. faecalis* (n = 35)	*E. faecium* (n = 28)
Tetracycline	47.4	65.7	53.6
Trimethoprim–sulfamethoxazole	26.3	—	—
Vancomycin	0	8.6	25.0
Linezolid	0	2.9	3.6

3.4 Multidrug and extensive drug resistance

A total of 239 out of 384 (62.2%) isolates were found to be MDR and 41 (10.7%) XDR according to consensus criteria (Magiorakos et al., 2012); no pandrug-resistant isolate was found. The highest MDR rates were seen in A. K. Baumanii (26/31;83.9%) pneumoniae (51/72; 70.8%), E. S. faecium and S. are the answer. golden staph MDR strains from hospitals were significantly more than community strains (196/277; 70.8% vs 43/107; 40.2%, OR 3.60, 95% CI 2.26-5.73, $\chi^2 = 30.91$, $p < 0.001$) (Table 5; Figure 4). By flies, we mean single flies. Out of a total of 432 flies, 268 (62.0%) possessed at least one MDR bacterium. A stark difference was observed in hospitals and communities, (226/318, 71.1% vs. 42/114, 36.8% OR 4.21, 95% CI 2.68-6.61; $p < 0.001$). the 41 XDR isolates were isolated from hospital flies, and 30 of them (73.2%) came from the ICUs.

Table 5. Multidrug resistance among bacterial isolates and flies by sampling origin.

Variable	Hospital sites	Community sites	OR (95% CI)	p value
MDR isolates, n/N (%)	196/277 (70.8)	43/107 (40.2)	3.60 (2.26–5.73)	< 0.001
XDR isolates, n/N (%)	41/277 (14.8)	0/107 (0)	—	< 0.001[a]
Flies carrying ≥1 MDR isolate, n/N (%)	226/318 (71.1)	42/114 (36.8)	4.21 (2.68–6.61)	< 0.001
ESBL-producing Enterobacterales, n/N (%)	90/150 (60.0)	24/58 (41.4)	2.13 (1.14–3.97)	0.016

[a] Fisher's exact test. OR, odds ratio; CI, confidence interval.

3.5 Molecular detection of resistance genes

The resistome of the 208 Enterobacterales isolates was mainly trafficked by ESBL genes. Specifically, blaTEM was detected in 121 isolates (58.2%), blaCTX-M group 1 in 109 (52.4%) and blaSHV in 64 (30.8%). The most common combination found in the isolates consisted of the co-occurrence of blaTEM and blaCTX-M genes. Sequencing revealed that the gene blaNDM-1 was confirmed in 11 K which less common but widely spread. pneumoniae (15.3%), seven P. 4 A. baumannii and three E's. (12.9%) 5 isolates K were found, (9 words). pneumoniae and an E. Five P. aeruginosa and two K. was used to characterize blaVIM. pneumoniae. Nineteen A sixty one point three percent of baumannii isolates carried blaOXA-23-like. This explains the high carbapenem resistance of this species. However, blaKPC was not detected. Enterobacterales harbored the class 1 integron marker intI1 in 41.8% of cases, and plasmid-mediated quinolone resistance determinants (qnrB/qnrS or aac(6')-Ib-cr) in 26.4% cases. Four isolates that were detected contained mcr-1 (two E. coli, two K. pneumoniae). Tests showed mecA present in all 17 cefoxitin resistant S. nine enterococci carried vanA-mediated resistance, while a single enteroccocis carried vanB-mediated resistance. The gene profile of ESBL in the fly isolates from the hospital (dominance of CTX-M-1 group) was similar to the reported profiles of clinical isolates in Iraqi hospitals (Hussein, 2018; Kareem et al., 2025).

Table 6. Prevalence of antimicrobial resistance genes among fly-associated isolates, detected by PCR.

Gene	*E. coli* (n = 96)	*K. pneumoniae* (n = 72)	*E. cloacae* (n = 22)	*P. mirabilis* (n = 18)	*P. aeruginosa* (n = 44)	*A. baumannii* (n = 31)
bla_{TEM}	52 (54.2)	46 (63.9)	12 (54.5)	11 (61.1)	—	—

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Gene	*E. coli* (n = 96)	*K. pneumoniae* (n = 72)	*E. cloacae* (n = 22)	*P. mirabilis* (n = 18)	*P. aeruginosa* (n = 44)	*A. baumannii* (n = 31)
bla$_{\text{CTX-M group 1}}$	47 (49.0)	41 (56.9)	11 (50.0)	6 (33.3)	—	—
bla$_{\text{SHV}}$	21 (21.9)	34 (47.2)	5 (22.7)	4 (22.2)	—	—
bla$_{\text{NDM-1}}$	3 (3.1)	11 (15.3)	2 (9.1)	0	7 (15.9)	4 (12.9)
bla$_{\text{OXA-48-like}}$	1 (1.0)	5 (6.9)	0	0	0	0
bla$_{\text{VIM}}$	0	2 (2.8)	0	0	5 (11.4)	0
bla$_{\text{OXA-23-like}}$	—	—	—	—	—	19 (61.3)
mcr-1	2 (2.1)	2 (2.8)	0	0	0	0
intI1	39 (40.6)	33 (45.8)	8 (36.4)	7 (38.9)	19 (43.2)	14 (45.2)

Values are n (%); —, not applicable. Among Gram-positive cocci, *mecA* was detected in 17/38 (44.7%) *S. aureus*, and *vanA*/*vanB* in 10/63 (15.9%) enterococci.

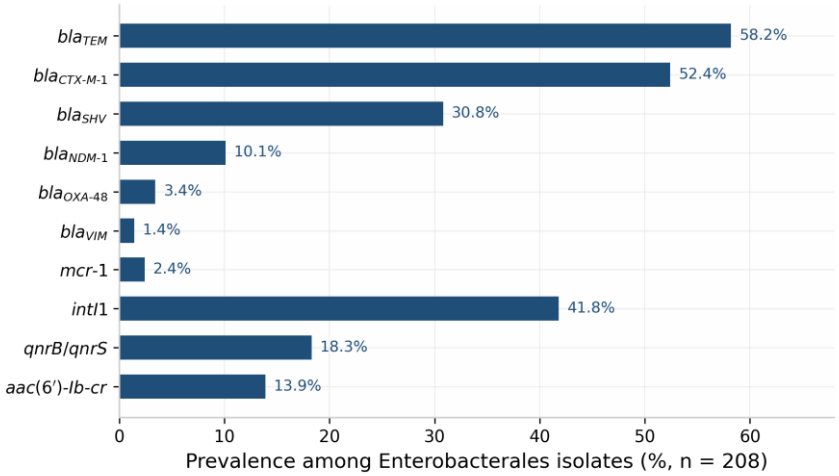

Figure 3. Prevalence of selected antimicrobial resistance genes among the 208 Enterobacterales isolates recovered from flies.

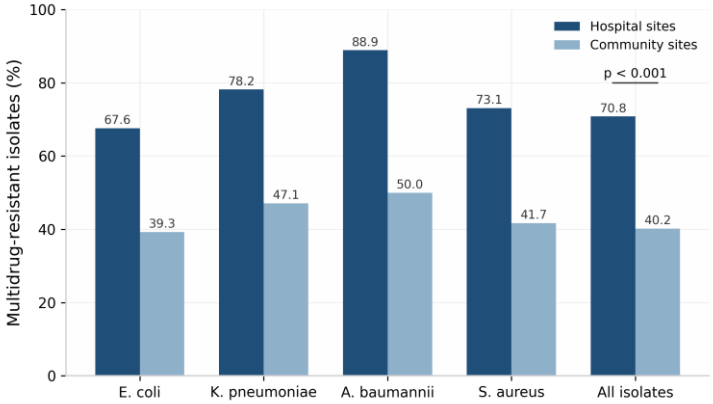

Figure 4. Proportion of multidrug-resistant isolates among hospital-derived versus community-derived isolates for the four most frequent pathogenic species and for all isolates combined (χ^2 test).

3.6 Carriage by fly species and body compartment

There was no significant difference in the carriage of MDR between M. This was particularly noted with M. domestica (63.0%) and the pooled blow fly/flesh fly group (59.8%) ($p = 0.52$), meaning all four capture tax are involved in transportation of bacteria resistant. The gut homogenates had a higher proportion of MDR isolates when compared to external washes (66.1% vs 59.3%; $p = 0.16$). Despite these findings not being significant overall, it was seen that 11.3% from gut were carbapenemase producers as compared to 6.9% from external washes ($p = 0.049$).

4. DISCUSSION

As far we know, this is the first combined entomological, phenotypic and molecular characterization of AMR bacteria from hospital-associated flies in Iraq. Three results clearly emerge. Almost all of the hospital fly species that were examined carried bacteria with clinical relevance. Also, more than 63 percent of all of these isolates were multidrug-resistant. Furthermore, there appeared to be a strong site-dependency of the resistance burden: while community flies carried multi-drug resistant organisms, hospital flies possessed more than double the likelihood of being associated with the organisms. Beyond that, only hospital flies were observed carrying XDR bacteria. Most of these flies were ICU flies. The fly isolates with resistance genes were dominated by blaCTX-M group 1 with more minor contributions of blaNDM-1, blaOXA-23-like, mecA and vanA. This is similar to the Iraqi clinical resistome reported (Ganjo et al., 2016, Hashim et al., 2026; Hussein, 2018; Ismail & Mahmoud, 2018; Kareem et al., 2025). All of these observations are consistent with flies acquiring resistant bacteria within the hospital environment and the potential of exporting them to the surrounding community.

The superiority of M. The data from this study (2018) is corroborated by previous surveys in other regions (Khamesipour et al., 2018; Ranjbar et al., 2016). This indicates strong synanthropy and tolerance of indoor environments. The existence of C. megacephala, L. sarcophaga and sericata species What these words mean work here means they prefer breeding in decaying organic matter and are attracted to wounds. Previous research has shown that megacephala carries ESBL production E. Punyadi et al., 2021 mentions e-coli in Thailand. The fact that MDR was evenly distributed across fly taxa suggests to us that species-targeted control would not be sufficient; the problem is ecological rather than taxonomic.

The resistance types reported here closely mirror those seen in the Iraqi clinics. In A. we have a carbapenem which is 67.7% resistant. The E. cloacae K. pneumoniae resistance of ceftazidime in K. pneumoniae and of cefotaxime in E. cloacae species. Bacteria sample strains K. coli 58.3%. Rates of S. pneumoniae (65.3%) are comparable with the levels observed in clinical isolates from Iraq (Abubaker & Anwar, 2023). The MRSA rate of S identified on flies The prevalence of S. aureus here was lower than the 74% national clinical figure (Alhilfi et al., 2026). Nevertheless, it indicates that flies do transport strains of direct nosocomial relevance. This is demonstrated for MRSA-carrying flies elsewhere (Achi et al., 2025). The four identified isolates featuring mcr-1 in Iraq mean the last-resort agent colistin is widely used in Iraqi hospitals where meropenem resistance is common. Moreover, mobile colistin resistance in an insect vector could accelerate its spread at the human–animal–environment interface (Gwenzi et al., 2021).

The gradient we saw at our hospital and community (60.0% vs. 41.4% ESBL carriage among all Enterobacterales) replicates the one reported from Ethiopia, where ESBL-producing Gram-negative bacteria were recovered from 67% of flies from the hospital but only 2% of flies trapped 1.5 km away (Tufa et al., 2020). Similarly, in Iran, with greater carriage of resistant bacteria by hospital flies than by flies from other environments (Nazari et al., 2017). The high community baseline in our study (41.4% ESBL) is likely due to environmental contamination with resistant Enterobacterales in Baghdad city where self-medication and antibiotic selling without prescriptions are well-documented (Al-Jumaili & Ahmed, 2024). Flies not only export hospital resistomes in these settings, but they also re-import community-acquired resistant organisms that may be introduced to susceptible in-patients. The fact that greater numbers of carbapenemase producers are found in the gut fractions than in external washes suggests that ingested bacteria remain in the intestinal tract. The significant presence of flies in the production environment may assist in the exchange of genes between resistant organisms (Bahrndorff et al., 2017; de Jonge et al., 2020; Fukuda et al., 2016).

The retrieval of WHO's high-priority pathogens. Operational implications arise directly from flies transferring bacteria, such as Acinetobacter baumannii, carbapenem-resistant Enterobacterales, and ESBL producers, from ICUs, kitchens, and waste areas. The house fly can disperse for a few kilometres while contaminating food, surfaces and wounds with its vomit and faeces (Khamesipour et al., 2018; Onwugamba et al., 2018). Moth flies breeding in hospital wastewater systems were already

implicated in the transmission of MDR within a European hospital (Rupprecht et al., 2020). In Iraq, currently, the use of infection prevention bundles is limited to hand hygiene and stewardship. Our data suggest that fly surveillance wherein flies are used as sentinels to sample the hospital resistome as proposed by Onwugamba et al. (2018) and Gwenzi et al. (2021)) should be incorporated into routine AMR monitoring, and that physical control measures (mesh screening of windows and vents, sealed waste management, routine drain maintenance) should be implemented in high-risk units.

There are limitations to this study. The cross sectional sampling cannot determine the direction of transmission between flies, patients and the environment, and whole-genome sequencing of paired fly, clinical and environmental isolates would need to be performed to demonstrate clonal links as achieved in the German moth fly study (Rupprecht et al., 2020). The generalization to other provinces of Iraq or seasons is likely to be constrained as sampling covered three hospitals in one city and one fly season. The true diversity within the fly microbiota particularly of anaerobes and other organics may be underrepresented in studies due to culture-based isolation. Additionally, phenotypic screening may have missed low-abundance resistant subpopulations. In conclusion, we did not measure the distance flies disperse within or beyond hospital premises; mark-release-recapture or genomic tracing studies would clarify the geographical scale of fly-mediated AMR dispersal.

5. CONCLUSIONS

In Baghdad, flies present in the hospitals act as mobile reservoirs and potential vectors of multidrug- and extensively drug-resistant bacteria including the WHO critical-priority pathogens (carrying blaCTX-M, blaNDM-1, blaOXA-23-like, mecA, vanA and mcr-1).

Potential Vectors of Drug-resistant Bacteria The substantial difference in resistance carriage between hospitals and communities suggests that flies pick up these microorganisms in these settings and definitely can transport them. In Iraq, infection prevention and control programmes should incorporate entomological surveillance and fly control as an important component of a One Health response to antimicrobial resistance. The sequencing of the genomes of flies, patients, and environmental isolates together over a period of time needs to be done in order to give some direction to the transmission and to check whether targeted fly control does bring down the burden of resistant infections acquired in hospitals.

DECLARATIONS

Ethics approval and consent to participate: The research study was approved by the institutional research ethics committee of the participating university and hospital administrations gave written permission for entomological sampling. No humans or patient data were utilized.

Consent for publication: Not applicable.

Availability of data and materials: The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no conflicts of interest.

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Authors' contributions: The study was designed by all authors, with equal participation in taking field samples, running laboratory experiments, analyzing data, and writing the manuscript.

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