Multidrug-Resistant Gram-Negative Pathogens Transported by Cockroaches in Urban Residential Areas of Katsina Metropolis

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Abstract

Cockroaches are significant pests in public health, known for spreading pathogens and causing foodborne illnesses. This study investigates their role in disseminating Gram-negative bacteria and multidrug resistance in Katsina metropolis. A total of 80 cockroaches were gathered from various locations including toilets, kitchens, sitting rooms, and bedrooms. Samples were incubated in tetrathionate broth for 24 hours, followed by serial dilutions and culturing on nutrient agar using the pour plate method. After 24-hour incubation at 37°C, selective media isolated bacterial species. Antibiotic susceptibility was assessed using the disk diffusion technique, and data analysis was performed with GraphPad Prism, with ($P < 0.05$) considered significant. Of the 115 bacterial species identified, 29 (25.2%) were from kitchens, 52 (45.2%) from toilets, 16 (13.9%) from bedrooms, and 18 (15.7%) from sitting rooms. Predominant isolates included *Klebsiella spp* (26.9%), *E coli* (22.6%), and *E aerogens*. (19.1%). High resistance rates were observed for cotrimoxazole (33.1%), while ciprofloxacin had the lowest resistance (1.1%). Among the isolates, 53.9% were multidrug-resistant, with 100% resistance seen in *Salmonella spp*, *A faecalis*, and *P vulgaris*. This study offers baseline data on gram-negative bacteria from cockroaches in Katsina homes, highlighting health risks and effective antibiotic treatments.

Keywords: Cockroaches, gram-negative bacteria, disk diffusion technique, MDR, non-MDR

Introduction

Cockroaches, ubiquitous pests in diverse environments including homes, food establishments, and healthcare facilities, pose significant health risks by contaminating food and transmitting various pathogens (Moges et al. 2016). They are known carriers of pathogens causing foodborne illnesses such as *Salmonella sp.*, *Shigella sp.*, *Staphylococcus aureus*, and *Bacillus cereus*, particularly in developing countries where poor food handling practices prevail (Ayana et al. 2015). Cockroaches thrive in unhygienic conditions, feeding indiscriminately on garbage and sewage, thereby facilitating the dissemination of human pathogens (Ayana et al. 2015; Pai et al. 2003). High densities of cockroaches are associated with health concerns due to allergies triggered by their bites and cuticular residues, exacerbating the transmission of disease-causing agents (Brenner and Kramer, 2019). Furthermore, these pests have been implicated in the global challenge of antibiotic resistance, harboring bacteria resistant to crucial antibiotics like cephalosporins and carbapenems (WHO, 2014; Wannigama et al. 2014).

Moving freely between various environments including human habitations, cockroaches play a significant role in the transmission of pathogenic bacteria responsible for diseases such as diarrhea, dysentery, cholera, leprosy, plague, and typhoid fever (Atiokena et al. 2017). Although not primary disease causes, their role as supplementary vectors underscores their impact on public health (Wilson et al. 2020). Given their habits of feeding on human waste and food, cockroaches contribute to the spread of disease-causing microorganisms, necessitating further microbiological studies to understand and mitigate these risks. Awareness of the pathogens carried by cockroaches is crucial for preventing foodborne illnesses, particularly in environments prone to poor hygiene practices. It is crucial to comprehend the microbiological characteristics of pathogens carried by

cockroaches to evaluate their disease-causing potential and establish efficient control strategies, particularly concerning gram-negative bacteria.

Material and methods

Study area, sample collection, and processing

The study was conducted in Katsina metropolis, the capital of Katsina state, Nigeria, one of the 36 states. The state has an estimated population of 10,368,500, with the predominant ethnic group being Hausa-Fulani, and the majority of residents practicing Islam. In Katsina metropolis, a total of 80 cockroaches were collected between September and October 2019. Among these, 20 were obtained from kitchens, 20 from toilets, and the remaining 40 from sitting rooms and bedrooms within households. Cockroaches were captured using sterile test tubes and immediately transported to the microbiology laboratory at Umar Musa Yar' adua University for bacteriological analysis within one hour of collection. Samples were stored at 4°C before analysis. Cockroaches were trapped by leaving containers open and through handpicking, where they were manually caught and transferred to containers. Analysis of the samples involved culture methods, including the use of an enrichment medium called tetrathionate broth for detecting *Salmonella spp.* and *Shigella spp*., in addition to nutrient broth prepared according to the manufacturer's guidelines.

Determining bacterial load

One milliliter of the content from each test tube containing cockroaches and enrichment media was transferred into a test tube with nine milliliters of sterile distilled water for serial dilution. This process was repeated until a dilution factor of 10^{-6} was achieved. Subsequently, 0.1 milliliters from the 10^{-4}, 10^{-5}, and 10^{-6} dilutions were transferred onto petri dishes, and nutrient agar was poured over them using the pour plate technique. After solidification, the plates were incubated at 37°C

for 24 hours. Following incubation, colonies were counted using a colony counter, and the counts were multiplied by the reciprocal of their dilution factor.

Bacterial isolation from the external surface of cockroaches

Cockroaches were washed vigorously in each test tube containing 10 mL of sterile normal saline before being transferred to secondary sterile test tubes. A loopful of each suspension was cultured on enrichment media was streaked onto various selective media including MacConkey agar, Shigella agar, Salmonella agar, and Cysteine Lactose Electrolyte Deficient agar using the streak plate technique. The plates were then incubated at 37°C for 24 hours. After incubation, colonies were observed and sub-cultured onto slants for further identification. Bacterial isolates were initially characterized by colony morphology and gram staining. Further identification involved standard biochemical procedures for Gram-negative bacteria.

Antimicrobial susceptibility testing

The antimicrobial susceptibility of the isolates to selected antibiotics was determined using the disc diffusion method on Mueller Hinton agar, following the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2011). Bacterial suspensions were spread evenly on the agar surface using a sterile L-shaped rod, adjusted to a concentration equivalent to 1.5×10^8 CFU/mL (0.5 McFarland standards) from 24-hour agar cultures. Antibiotic discs were placed equidistantly on the agar plates using sterile forceps and incubated at 37°C for 24 hours after a pre-diffusion period of 60 minutes. Bacterial isolates resistant to three or more antibiotic classes were classified as multidrug-resistant (MDR) strains, following criteria described by Magiorakos et al. (2012).

Analysis of data and presentation of results

Statistical analysis was performed using GraphPad Prism Version 11.0 software. Data were derived from three independent experiments. One-way and two-way ANOVA followed by Tukey's post hoc test was used to assess more than 2 variables while the student t-test was for 2 variables. A p-value < 0.05 was considered statistically significant.

Result

The bacterial population found on the surface of the cockroaches varied according to the site of collection of the average bacterial load (Figure 1). The result analysis indicates that the sample collected from the toilet had a higher average bacterial load of 7.9×10^8 cfu/ml. In comparison, the least count was obtained from the kitchen 2.1×10^8 cfu/ml. Still, there is no statistically significant difference observed in the bacterial count between sites of collection as indicated through one-way ANOVA ($P<0.05$). Our bacterial load indicates similar contamination levels across locations.

A total of 115 bacterial species were isolated from the kitchen, toilet, sitting room, and bedroom. The toilet had the highest number of bacterial isolates at 52 (45.2%), while the bedroom had the fewest at 16 (13.9%) while the kitchen had the second highest number of isolates (25.2%), followed by the sitting room (15.7%) and the bedroom (13.9%). (Table 1). *Klebsiella spp*. was the most common isolate with 31 (26.9%) occurrences, followed by *E. coli* 26 (22.6%) and *E aerogenes* 22 (14.8%). The least common isolates were *P. vulgaricus* 01 (0.9%) and *A. faecalis* with 02 (1.7%). Specifically, *P. aeruginosa* was the most common isolated in kitchen 8 (47.1%), *E. coli* in the toilet with 16 (61.5%), *Klebsiella spp.* in the sitting room with 5 (16.1%), and *Klebsiella spp*. in the bedroom with 6 (19.4%). *Klebsiella spp* and *E. coli* were the most common isolates overall, making up 26.9% and 22.6% of the total isolates, respectively. *A. feacalis* and *P. vulgaricus* were the least common, each found only in the toilet with very low occurrence. These findings indicate that cockroaches in residential areas can carry a variety of bacterial species, with

the highest contamination levels found in toilets. The toilet had the highest number of bacterial isolates, suggesting it is the most contaminated site among the locations tested. The presence of different bacterial species in various locations highlights the widespread contamination risk posed by cockroach infestations in homes.

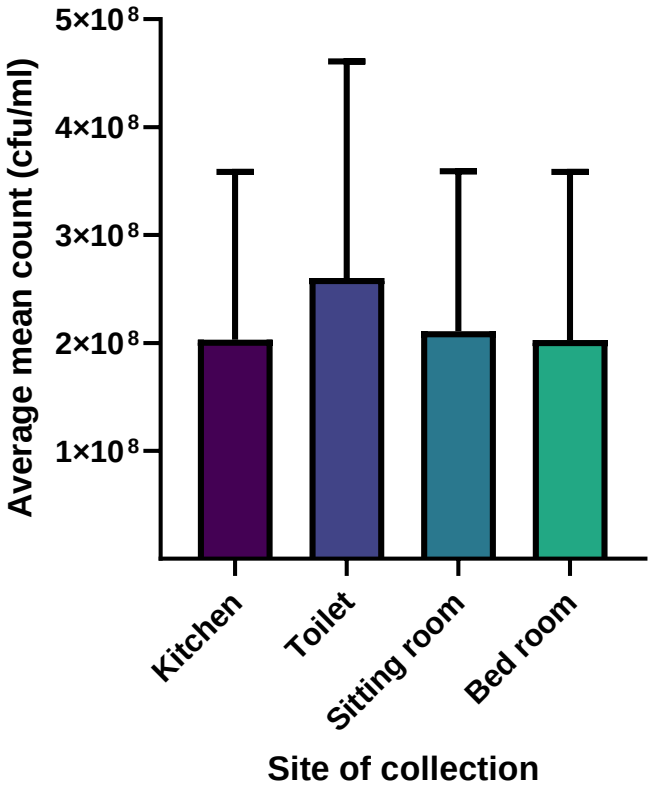

Figure 1: Average bacterial count in the surface body of the cockroaches based on the site of collection expressed in colony forming unit per milliliter (cfu/mL).

Table 1: Bacterial isolates identified from the cockroaches' body surface in the respective locations of the household of Katsina metropolis

Bacterial isolates	Location				Total
	Kitchen	Toilet	Sitting room	Bedroom	
Salmonella spp	0 (0.0)	2 (66.7)	1 (33.3)	0 (0.0)	3 (2.6)
Shigella spp	2 (15.4)	6 (46.2)	3 (23.1)	2 (15.4)	13 (11.3)
Klebsiella spp	8 (25.8)	12 (38.7)	5 (16.1)	6 (19.4)	31 (26.9)
E coli	3 (11.5)	16 (61.5)	4 (15.4)	3 (11.5)	26 (22.6)
A feacalis	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	2 (1.7)
P aerogenosa	8 (47.1)	7 (41.2)	1 (5.9)	1 (5.9)	17 (14.8)
E aerogens	8 (36.4)	6 (27.3)	4 (18.2)	4 (18.2)	22 (19.1)
P vulgaricus	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	1 (0.9)
Total	**29 (25.2)**	**52 (45.2)**	**18 (15.7)**	**16 (13.9)**	**115(100.0)**

The antibiotic resistance rates and patterns of bacterial isolates from cockroach body surfaces are presented in Table 2. For *Salmonella spp*., the highest resistance was to Gentamicin (52.6%), while no resistance was observed for Sulfonamides. *Shigella spp*. showed the highest resistance to Gentamicin and Cephalosporins (21.4% each), with no resistance to Amoxicillin. *Klebsiella spp.* had the highest resistance to Cephalosporins (22.2%), followed by Gentamicin (15.6%). *E. coli* showed the most resistance to Cephalosporins (20.4%), while *A. feacalis* had the highest resistance to Trimethoprim-sulfamethoxazole (30.8%). *P. aeruginosa* exhibited varied resistance, with the highest rates for Gentamicin, Cephalosporins, and Ofloxacin (13.8% each). *E. aerogenes* had the highest resistance to Sulfonamides (32.0%), and *P. vulgaricus* was most resistant to Ofloxacin (50.0%). Our findings suggest that bacteria show diverse resistance patterns, emphasizing the importance of personalized treatment strategies for specific pathogens and the potential risk of resistant infections spreading in residential settings.

The overall resistance rate shows that bacterial isolate with MDR pattern had a higher significant proportion compared to Non-MDR (Figure 2). Additionally, Figure 3 presents the percentage of MDR and non-MDR bacterial isolates for various bacterial isolates. The bar graph compares the proportions of MDR and non-MDR isolates for each bacterial species. The high prevalence of MDR strains among the tested bacterial isolates, particularly *Salmonella spp., Shigella spp., A. faecalis*, and *P. vulgaricus*, where nearly all isolates are MDR. Other species like *P. aeruginosa* show a higher proportion of non-MDR isolates. The results indicate that multidrug resistance (MDR) is significantly more prevalent among almost all isolates. The exceptions are *E. aerogenes*, which has a significantly higher percentage of non-MDR isolates, and *E. coli*, where there is no significant difference in the proportion of MDR and non-MDR isolates.

Table 3 presents the antibiotic resistance patterns of various bacterial isolates collected from different points within households. *Salmonella spp* most isolates from the toilet showed multidrug resistance (MDR) (66.7%), significantly higher than in other locations. *Shigella spp* significant levels of MDR were observed in isolates from the toilet (38.5%) compared to other locations, although not statistically significant. *Klebsiella spp* MDR proportion was notable in the toilet (32.3%), but not statistically significant across locations. *E coli* MDR proportion was significantly higher in the kitchen (5.5%) compared to other locations. *Alcaligen feacalis* isolates from the toilet showed complete resistance (100%), which was significantly higher than other locations ($p=0.00544$). *Pseudomonas aerogenosa* no significant difference in MDR proportion was observed across different locations. *Enterobacter aerogens* MDR proportion was significantly higher in the kitchen (22.7%) compared to other locations ($p=0.02112$). *Proteus vulgaricus* isolates from the toilet showed complete resistance (100%), significantly higher than other locations.

Table 2: Resistance rate of Bacterial isolates identified from cockroaches in the household

	Antibiotics Tested									
Bacterial isolate	**S**	**CN**	**PN**	**CEP**	**PEP**	**AU**	**NA**	**SXT**	**CPX**	**OFX**
Salmonella spp	0 (0.0)	10 (52.6)	1 (15.2)	2 (10.5)	1 (5.2)	NT	1 (5.2)	3 (15.7)	1 (5.2)	NT
Shigella spp	2 (7.1)	6 (21.4)	2 (7.1)	6 (21.4)	1 (3.6)	0 (0.0)	2 (7.1)	3 (10.7)	4 (14.2)	2 (7.1)
Klebsiella spp	4 (8.9)	7 (15.6)	4 (8.9)	10 (22.2)	3 (6.7)	6 (13.3)	2 (4.4)	3 (6.7)	6 (13.3)	NT
E coli	6 (12.2)	8 (16.3)	4 (8.1)	10 (20.4)	5 (10.2)	2 (4.4)	1 (2.0)	NT	5 (10.2)	8 (16.3)
A feacalis	1 (7.7)	NT	0 (0.0)	2 (15.4)	3 (23.1)	NT	NT	4 (30.8)	2 (15.4)	1 (7.7)
P aerogenosa	3 (10.3)	4 (13.8)	3 (10.3)	4 (13.8)	4 (13.8)	2 (6.9)	1 (3.4)	1 (3.4)	3 (10.3)	4 (13.8)
E aerogens	8 (32.0)	6 (24.0)	2 (8.0)	2 (8.0)	1 (4.0)	2 (8.0)	1 (4.0)	NT	2 (8.0)	1 (4.0)
P vulgaricus	0 (0.0)	0 (0.0)	0 (0.0)	1 (16.7)	1 (16.7)	1 (16.7)	0 (0.0)	NT	0 (0.0)	3 (50.0)
Total	**24 (20.9)**	**41 (35.7)**	**16 (13.9)**	**37 (32.2)**	**20 (17.4)**	**13 (11.3)**	**8 (6.9)**	**14 (12.2)**	**23 (20.0)**	**20 (17.4)**

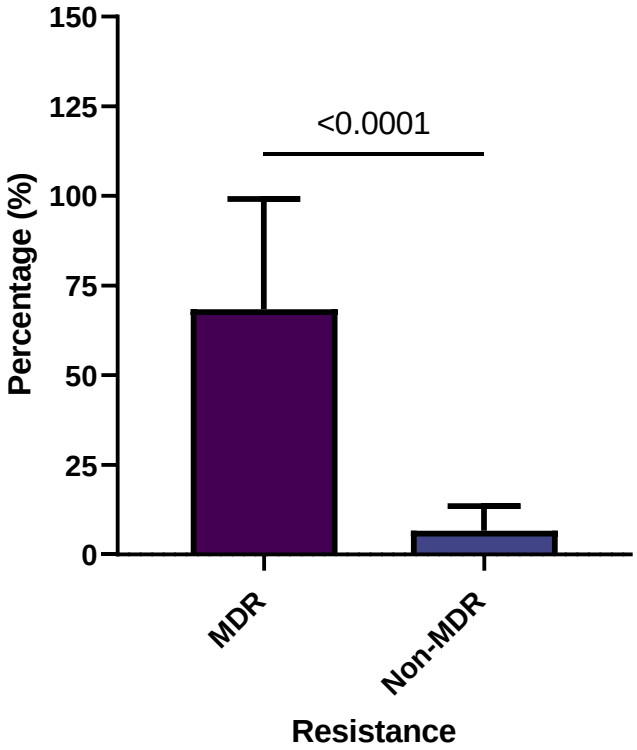

Figure 1: Proportion of multidrug resistance pathogens (MDR) and non-multidrug resistance pathogens (Non-MDR) among the bacterial isolates.

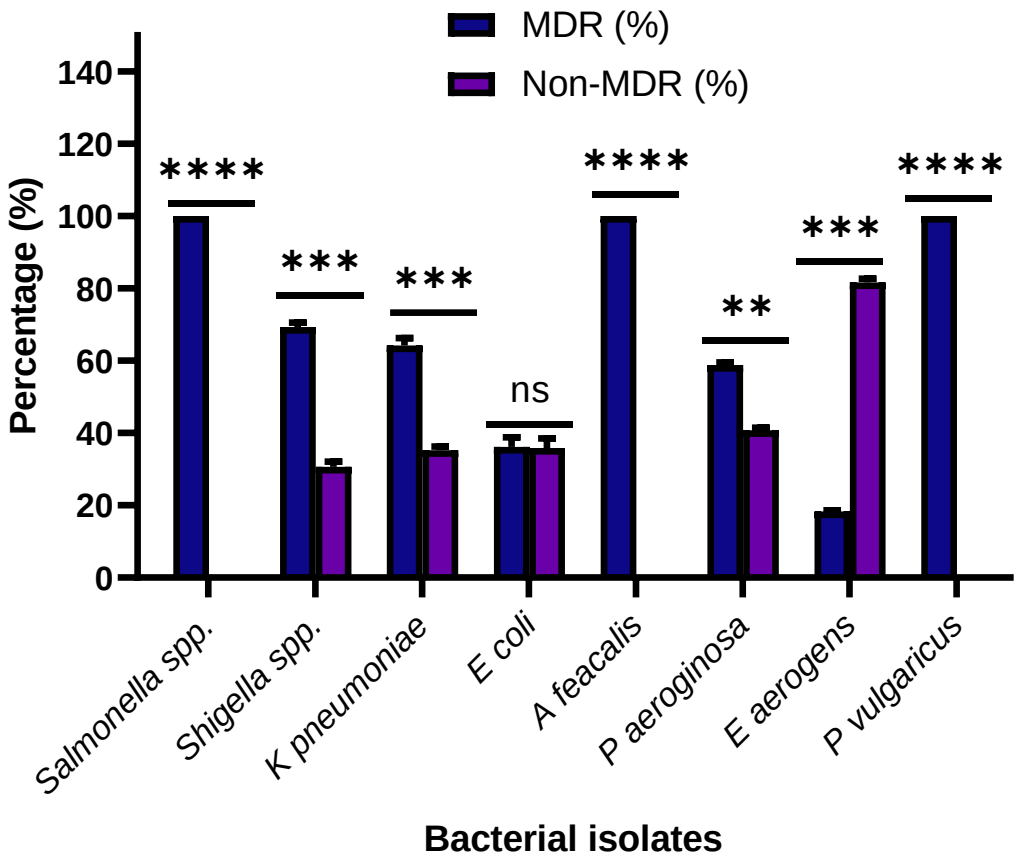

Figure 3: Proportion of MDR and non-MDR isolates identified from the body surface of cockroaches

Table 3: The proportion of MDR and Non-MDR isolates in cockroaches from different points

Bacterial isolates	Points of collection	MDR	Non-MDR	P-value
Salmonella spp	Kitchen	0 (0.0)	0 (0.0)	0.00211
	Toilet	2 (66.7)	0 (0.0)	
	Sitting room	1 (33.3)	0 (0.0)	
	Bedroom	0 (0.0)	0 (0.0)	
Shigella spp	Kitchen	2 (15.4)	0 (0.0)	0.08923
	Toilet	5 (38.5)	2 (15.4)	
	Sitting room	1 (7.7)	2 (15.4)	
	Bedroom	1 (7.7)	0 (0.0)	
Klebsiella spp	Kitchen	4 (12.9)	3 (9.7)	0.12218
	Toilet	10 (32.3)	2 (6.5)	
	Sitting room	4 (12.9)	4 (12.9)	
	Bedroom	2 (16.5)	2 (6.5)	
E coli	Kitchen	2 (5.5)	3 (8.3)	0.00111
	Toilet	8 (22.2)	3 (8.3)	
	Sitting room	1 (2.8)	4 (11.1)	
	Bedroom	2 (5.6)	3 (8.3)	
Alcaligen feacalis	Kitchen	0 (0.0)	0 (0.0)	0.00544
	Toilet	2 (100.0)	0 (0.0)	
	Sitting room	0 (0.0)	0 (0.0)	
	Bedroom	0 (0.0)	0 (0.0)	
Pseudomonas aerogenosa	Kitchen	6 (35.3)	4 (23.5)	0.67510
	Toilet	4 (23.5)	3 (17.6)	
	Sitting room	0 (0.0)	1 (5.9)	
	Bedroom	0 (0.0)	1 (5.9)	
Enterobacter aerogens	Kitchen	1 (4.5)	5 (22.7)	0.02112
	Toilet	3 (13.6)	7 (31.8)	
	Sitting room	0 (0.0)	3 (13.6)	
	Bedroom	0 (0.0)	3 (13.6)	
Proteus vulgaricus	Kitchen	0 (0.0)	0 (0.0)	0.00001
	Toilet	1 (100.0)	0 (0.0)	
	Sitting room	0 (0.0)	0 (0.0)	
	Bedroom	0 (0.0)	0 (0.0)	

Discussion

Our study identified various gram-negative bacterial species on the body surfaces of household cockroaches in the Katsina metropolis, with these bacteria spreading to different areas of homes including kitchens, toilets, sitting rooms, and bedrooms. Previous research on cockroach-pathogenic bacteria in residential settings among students, teachers, and low-income employees (Wannigama et al., 2014) aligns with our findings. Literature also supports that cockroaches harbor a diverse array of bacteria on their external surfaces, influenced by their habitat and feeding behaviors (Turki Jalil et al., 2023).

Additional research has consistently shown significant bacterial contamination linked to cockroaches in diverse environments, from households to healthcare facilities (Guzman and Vilcinskas, 2020; Turki Jalil et al., 2023). Cockroaches acquire pathogens from their environment, including feces and contaminated surfaces, posing potential health risks such as foodborne illnesses and infections. The higher prevalence of bacterial isolates in toilets compared to other areas underscores the role of sanitation practices and the potential for fecal contamination in contributing to bacterial diversity. Studies have shown that toilets can serve as reservoirs for a wide range of bacteria, including opportunistic pathogens like *Klebsiella spp*. and *E. coli*, which thrive in moist and nutrient-rich environments. The absence of a statistically significant difference in bacterial counts across different sites in this study contrasts with the findings of Turki Jalil et al. (2023). While bacterial load variation was observed, factors such as sample size, environmental conditions, and local hygiene practices could influence these results.

Furthermore, researches might explore seasonal variations, specific bacterial species identified, and the implications for public health interventions targeting cockroach control and sanitation practices in urban residential settings. The high occurrence of *E. coli* and *Klebsiella spp*. found in

cockroaches in this study is consistent with the findings of Davari et al. (2023). Similarly, a study conducted in Sokoto identified *E. coli* as the most prevalent isolate (Ibrahim et al., 2017). The predominance of *Klebsiella spp.* and *E. coli* highlights these organisms as common inhabitants of indoor environments and potential pathogens implicated in various infections. Additionally, research conducted in Iraq identified *Klebsiella spp., Pseudomonas spp., Proteus spp., E. coli, Shigella sonnei,* and *Salmonella spp.* as prevalent isolates. The detection of various bacteria on the external body parts of cockroaches in our study is consistent with findings by Turki Jalil et al. (2023). Moreover, a study conducted in Ibadan by Adejumo et al. (2016) isolated several gram-negative bacteria, including *Pseudomonas spp., Proteus spp., Klebsiella spp., Salmonella spp., Escherichia coli, Serratia marcescens, Shigella spp., Pseudomonas oryzihabitans, Klebsiella pneumoniae, Salmonella enterica,* and *Proteus vulgaris.* Collectively, these findings support the idea that household environments, including kitchens and toilets, harbor diverse bacterial species often associated with human activities and hygiene practices.

These cockroaches could act as mobile reservoirs for the identified gram-negative bacteria, potentially initiating new outbreaks of life-threatening septicemia infections. Notably, cockroaches are carriers of severe diseases, including *Salmonella Typhi,* which causes Typhoid. They are also associated with dysentery, which causes severe diarrhea with possible bleeding, and gastroenteritis from food poisoning, resulting in diarrhea, nausea, vomiting, and abdominal pain. These diseases significantly impact public health and lead to substantial economic losses and fatalities. Cockroaches are linked to gram-negative bacteria due to their habitats and feeding habits, which involve living in and consuming human and animal excreta, garbage, and similar materials.

The antibiotic susceptibility analysis of the bacterial isolates showed a common pattern of multiple drug resistance to the antibiotics used. These findings align with previous studies that have

documented antibiotic resistance in bacteria associated with cockroaches, reflecting the potential role of these insects in harboring and disseminating multidrug-resistant pathogens in residential environments (Taha et al. 2014). Effective surveillance and management strategies are crucial to mitigate the spread of antibiotic-resistant bacteria associated with cockroaches and to preserve the efficacy of antibiotics in clinical practice. The findings reveal a notable prevalence of multidrug-resistant (MDR) bacterial isolates from cockroach body surfaces, consistent with existing literature on antibiotic resistance in environmental pathogens. This aligns with concerns raised in studies emphasizing the role of cockroaches in harboring and disseminating resistant pathogens (Vazirianzadeh et al., 2014). The distribution of MDR and non-MDR isolates among specific bacterial species such as *Salmonella spp.*, *Shigella spp.*, *A. faecalis,* and *P. vulgaricus* exhibit predominantly MDR patterns, with nearly all isolates demonstrating resistance to multiple antibiotics. In contrast, *P. aeruginosa* shows a higher proportion of non-MDR isolates, indicating variable resistance profiles within bacterial species associated with cockroaches.

These findings underscore the urgent need for effective surveillance and control measures to mitigate the spread of MDR bacteria facilitated by cockroaches in residential environments. Our findings show *Salmonella spp*. exhibited a strikingly high multidrug resistance (MDR) rate in isolates from toilets, emphasizing the potential for significant public health implications (Taha et al. 2014). Similarly, *Shigella spp*. showed substantial MDR levels in toilet isolates, albeit not statistically significant compared to other sites, suggesting localized resistance dynamics (Pai et al., 2003). *Klebsiella spp*. demonstrated notable MDR proportions in toilets, highlighting a trend of resistance in this bacterial species within household environments (Sharma et al., 2023). Conversely, *E. coli* exhibited a significantly higher MDR proportion in kitchen isolates, indicating potential niche-specific resistance patterns influenced by environmental factors (Sharma et al.,

2023). *Alcaligenes faecalis* and *Proteus vulgaricus* isolates from toilets displayed complete resistance to tested antibiotics, emphasizing the challenges posed by antibiotic-resistant bacteria in specific household locations (Huang, 2020). *Pseudomonas aeruginosa* showed no significant difference in MDR proportions across locations, suggesting a uniform resistance profile irrespective of household site. *Enterobacter aerogenes* demonstrated significantly higher MDR proportions in kitchen samples, further highlighting location-specific variations in antibiotic resistance among bacterial isolates (Davin-Regli and Pages, 2015). These findings underscore the need for tailored antimicrobial stewardship strategies and enhanced hygiene practices to mitigate the spread of multidrug-resistant bacteria within residential settings.

Conclusion

In conclusion, this study stresses the pivotal role of household cockroaches in harboring and disseminating various gram-negative bacterial species within urban residential settings in the Katsina metropolis. The findings demonstrate a consistent pattern of bacterial contamination across different areas of homes, with toilets housing the most bacterial species, notably Klebsiella spp. and E. coli, likely due to inadequate sanitation practices. The study also underlines the potential health risks associated with cockroaches, including the transmission of foodborne illnesses and infections. The antibiotic susceptibility analysis further highlights a troubling trend of multidrug resistance among bacterial isolates, emphasizing the urgent need for effective surveillance, control measures, and enhanced hygiene practices to mitigate the public health impact of these pathogens. Effective antimicrobial stewardship is vital in addressing the spread of multidrug-resistant bacteria carried by cockroaches and maintaining antibiotic efficacy.

Author contributions

Hayatuddeen Muhammad Rumah: Conceptualisation, Methodology, Validation, Writing - Review & Editing, Resources and Supervision, Formal Analysis, Investigation, Data Curation. Maharazu Sani: Writing - Original Draft. Fatima Mukhtar: Resources and Visualization.

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Disclosure statement

The authors declare that there are no conflicts of interest regarding the publication of this manuscript. The research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data available statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

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