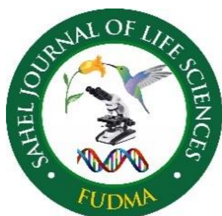

Sahel Journal of Life Sciences FUDMA (SAJOLS)
June 2026 Vol. 4(2): 15-24
ISSN: 3027-0456 (Print)
ISSN: 1595-5915 (Online)
DOI: https://doi.org/10.33003/sajols-2026-0402-03

Research Article

Antimicrobial Potential of Bacteria Isolated from Cockroaches in Polluted Environments

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ABSTRACT
The emergence of antimicrobial resistance (AMR) has posed an imminent threat to the discovery of new antimicrobial agents with alternative biological origins. The microbiota associated with insects is now of considerable interest as producers of bioactive metabolites, as they are exposed to diverse microbial communities in their habitat. This study examined the antimicrobial activity of cockroach bacteria, which survive in polluted environments (sewage systems, refuse dumps, and waste disposal sites). The cockroaches were dissected, bacterial isolates were collected from the gut, cultured and then screened against *Enterococcus faecium, Klebsiella pneumoniae* and *Acinetobacter baumannii*. Seven bacterial isolates were identified using 16S rRNA gene sequences, most of which belonged to the genera Bacillus and Enterobacter. Antimicrobial screening revealed that some isolates were inhibitory, and their ethyl acetate extracts showed high antimicrobial activity. Isolate WP2 ethyl extract, which was identified as *Bacillus altitudinis*, exhibited a 24 mm inhibition zone against *E. faecium*, 20 mm against *A. baumannii*, and 16 mm against *K. pneumoniae.* LC-MS identified bioactive compounds, including celastramycin A, cefditoren pivoxil, meclocycline, and sericetin, which exhibit antibacterial, antiviral, and antioxidant effects. Such findings demonstrate that bacteria coevolved with cockroaches, and can serve as an alternative source of new antimicrobial drugs, and that insect microbiomes can be drug-discovery worthy.

Keywords: Cockroach; Polluted environments; Bacteria, Public Health; Secondary metabolites

Citation: Haruna, E., James, S.A., & Maiangwa, J.J. (2026). Antimicrobial Potential of Bacteria Isolated from Cockroaches in Polluted Environments. *Sahel Journal of Life Sciences FUDMA*, 4(2): 15-24. DOI: https://doi.org/10.33003/sajols-2026-0402-03

INTRODUCTION

One of the greatest threats to global health in the twenty-first century has become antimicrobial resistance (AMR). Multidrug-resistant pathogens have emerged and become a major problem, leading to the loss of effectiveness of conventional antibiotics and making it hard to cure common infections. Antimicrobial resistance has developed due to the use and misuse of antimicrobial agents in clinical medicine, agriculture, and animal production, resulting in increased morbidity, mortality, and medical costs worldwide (World Health Organisation, 2023).

The threat of antibiotic resistance has become increasingly serious, making it imperative to find new antimicrobial substances in other biological sources. In the past, most antibiotics have been derived from soil microorganisms, particularly *Streptomyces* species. However, an extensive exploration of these conventional sources has led to a decrease in the number of new antimicrobial compounds obtained. As a result, there has been a slow but steady increase in interest among the scientific community in the study of unusual ecosystems and host-associated microbiomes as novel sources of bioactive molecules.

Insects are the most fruitful and diversified group of life on Earth, and they favour complicated communities of microbes that are supposed to contribute to host physiology, host immunity and host adaptation. These symbiotic microbes usually have a second metabolite which serves their hosts in defence against pathogenic organisms and competitors. The insect microbial gut regulates host health by modulating the immune response and producing antimicrobial agents that eliminate pathogenic bacteria (Engel and Moran, 2019).

Cockroaches are also among the most evolutionarily advanced insects, as well as tough, and can survive in environments with waste, such as sewage systems, refuse dumps, and garbage disposal sites (Bell *et al*., 2007; Moran *et al*., 2009). High microbial diversity and competition for resources that favour the selection of microorganisms capable of developing antimicrobial molecules are the biomarkers of these environments. As a result, cockroaches and their associated microbiota may also serve as microbial reservoirs capable of generating bioactive metabolites (Akbar *et al*., 2018; Ali *et al*., 2017).

Although cockroaches have long been the subject of study due to their role in the spread of pathogenic microorganisms, microbiomes and ecological roles are now being studied. The gut microbiota of cockroaches, as demonstrated in earlier investigations, performs important functions in digestion, detoxification, immune response, and microbial protection (Gladkikh *et al*., 2025). In addition, antimicrobial peptides and insect-associated bacteriocins have been postulated to be produced by bacteria, thereby inhibiting pathogenic bacteria (Zhang *et al*., 2025).

Molecular microbiology and metabolomics have recently emerged as powerful tools for exploring insect-associated microorganisms in antimicrobial discovery. Recent advances in high-throughput sequencing technologies have shown that insect microbiomes harbour numerous biosynthetic gene clusters, which encode antimicrobial secondary metabolites (Turner *et al*., 2024). These findings demonstrate that insect-based bacteria are a poor source of novel antimicrobial samples.

Irrespective of such developments, other studies have not largely examined the microorganisms that inhabit cockroaches living in polluted environments in as large numbers as a potential source of antimicrobial metabolites. Most historical studies have focused on cockroaches as mechanical carriers of pathogens rather than as vectors of useful microorganisms that have pharmaceutical applications.

Therefore, the present study aims to isolate, characterise, and assess the antimicrobial activity of bacteria harboured by cockroaches living in polluted environments.

MATERIALS AND METHODS

Study Area and Collection of Samples

Cockroach samples were collected from different polluted environments (sewage systems, refuse dumps, and waste disposal sites). These environments have been chosen because they are abundant in organic waste and microbial diversity, which are likely to harbour microorganisms capable of producing antimicrobial compounds. At each location, 10 cockroaches were trapped with sterile traps and forceps and transferred to sterile containers immediately. The samples were moved to the laboratory within 2 to 4 hours of collection for further processing. After being killed in a sterile jar containing chloroform, the specimens were morphologically identified using standard entomological keys (Dong *et al*., 2022). The cockroaches were identified as either *Periplaneta americana* or *Blattella germanica* at the Department of Biological Sciences, Kaduna State University.

Surface Sterilisation and Dissection

To isolate microorganisms from the cockroach gut, surface sterilisation was performed first, as previously described (Gebreyohans *et al*., 2024). Concisely, cockroaches were washed with sterile distilled water and dipped in 70% ethanol for approximately 2 minutes to kill any transient microorganisms on the surface. The specimens were then rinsed again with sterile distilled water to remove residual ethanol. The cockroaches were dissected under aseptic conditions using sterile dissecting instruments. The digestive tract was carefully removed and transferred into sterile test tubes with physiological saline solution. The gut tissues were homogenised with sterile glass homogenisers to liberate the associated microorganisms.

Isolation of Microorganisms

The homogenised gut samples were diluted serially in sterile physiological saline solution. Aliquots of the diluted samples were spread-plated on Nutrient agar. The inoculated plates were incubated at 37 ^{0}C for 18 hr. Colonies with morphological differences were picked and subcultured repeatedly on fresh agar plates to obtain pure isolates. The purified isolates were stored on agar slants for subsequent identification and antimicrobial screening.

Primary Screening of Antimicrobial Activity

The antimicrobial effects of the isolated microorganisms were evaluated using the spread-and-patch method as previously described by Akbar *et al*. (2018). The clinically relevant bacterial pathogens that were used as test organisms included *Enterobacter faecium, Acinetobacter baumannii*, and *Klebsiella pneumoniae*. Fresh cultures of the test organisms were prepared and adjusted to a turbidity equivalent to 0.5 McFarland standard. Sterile Nutrient agar plates were spread with the test organisms with sterile swabs to produce a uniform bacterial lawn. Isolates were patched onto the pathogens, and the plates were incubated at 37°C for 24 hours. After incubation, antimicrobial activity was measured as the diameter of the inhibition. The results were considered positive if a zone of inhibition was observed and were used to assess the antimicrobial potential of the isolates.

Bacterial fermentation

Bacteria that inhibited the test organisms' growth from the primary screening were used for this stage. Concisely, a colony of fresh cultures was inoculated into 500 mL sterile Nutrient broth and incubated at 37 ^{0}C with shaking (150-200 rpm) for 7 days. The fermented cultures were centrifuged at 8,000-10,000 x g for 10 min to separate the bacterial cells and the broth. The cell-free supernatant was collected and used for secondary metabolite extraction. Production of secondary metabolites by fermentation of bacterial isolates was carried out as previously described by Demain and Sanchez (2009).

Secondary Metabolites extraction with ethyl acetate

The ethyl acetate extract was used to isolate secondary metabolites from the fermentation broth, using the liquid-liquid extraction procedure described earlier (Strobel and Daisy, 2003). Ethyl acetate (1:1, v/v) was added to the fermentation broth and mixed; the extracellular metabolites were extracted within 15-20 minutes. The organic phase was left to separate, and it was collected carefully. The extraction was done twice to get maximum recovery of the metabolites. The collected ethyl acetate fractions were further concentrated under reduced pressure in a rotary evaporator at 40 ^{0}C to obtain crude extracts, which were stored at 4 ^{0}C until the next use.

Preparation of extract-impregnated disks

Dimethyl sulfoxide (DMSO) was used to dissolve the crude to obtain a 100 mg/mL concentration. The disks were impregnated to achieve a 10mg and dried under sterile conditions to remove residual solvent as previously described by Balouiri *et al*. (2016).

Antibacterial activity of the secondary metabolites

The agar disc diffusion was used to determine the antibacterial activity of the crude extracts. Test bacterial strains were cultured overnight and adjusted to a 0.5 McFarland standard (approximately 1 × 10^8 CFU/mL). Mŭeller-Hinton agar plates were prepared, and the bacterial suspension was spread using sterile cotton swabs. The sterile forceps were used to place the extract-impregnated disks on the agar surface. The plates were incubated at 37 ^{0}C for 18-24 h, and the diameter of the zone of inhibition (mm) around each disc was measured to determine antibacterial activity. The experiment was performed three times, and the average zone diameter of inhibition was recorded for each. The screening was performed as described in the Clinical and Laboratory Standards Institute and other antimicrobial screening (Balouiri *et al*., 2016).

DNA Extraction and 16S rRNA Gene Amplification

The DNA of all six active bacterial isolates was extracted using the UltraClean Microbial DNA Isolation Kit according to the manufacturer's instructions (Mo Bio Laboratories, Carlsbad, CA, USA). Briefly, 1.8 mL of an overnight bacterial culture was centrifuged at 10,000 x g for 30 seconds, and the pellet was resuspended in MicroBead solution—mechanical lysis and subsequent purification with Solutions MD1-MD4, followed by DNA elution with 50 uL Solution MD5. Moreover, the 16S rRNA gene, approximately 1450 bp, was amplified with primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′), and 765R (5′-CTGTTTGCTCCCCACGCTTTC-3′);
704F(5′-GTAGCGGTGAAATGCGTAGA-3′), and 1492R (5′TACGGYTACCTTGTTACGACTT-3′). PCR reactions (50 µL) contained 2X Master Mix containing

Pyrococcus furiosus DNA polymerase, primers and RNase-free water and ~10 ng genomic DNA. PCR cycling profile: denaturation time was 1 min at 94 ^{0}C, followed by 35 cycles of denaturation time 1 min at 94 ^{0}C, 55 ^{0}C, and 72 ^{0}C, and a final extension time of 10 min at 72 ^{0}C. purification of the amplicons was performed using the NucleoSpin Gel and PCR Clean-up Kit (Macherey-Nagel, USA), and sequencing was performed by Inqaba Biotechnical Industries (Pty) Ltd. The quality and concentration of genomic DNA and amplified 16S rRNA gene were verified with 1% agarose gel electrophoresis.

Bacterial Identification

The pair-wise sequence alignment (nucleotide) algorithm has been used to align the two fragments of the sequenced 16S rRNA gene (1300-1450 bp) in their entirety (Li *et al*., 2015). The sequences were then aligned, followed by a BLAST suite (Johnson *et al*., 2008) search program to identify the isolates with high similarity to the species.

LC-MS analysis of WP 2 crude extracts

Untargeted metabolomics analysis (LC-MS) was performed on a PerkinElmer FX-15 ultra-high-performance liquid chromatography (UHPLC, PerkinElmer Incorporation, Massachusetts, USA) with a Sciex 3200 QTRAP hybrid linear ion trap quadrupole mass spectrometer and an autosampler vacuum degasser, analytical column (Phenomenex Synergy RP C18, 100A, 100mm x 3uM x 2. The active extract of WP2 in ethyl acetate (1000 µg/mL, three technical replicates per biological sample) was injected directly into the 250 µL syringe. The mobile phase, Solvent A, was made up of water with 0.1% formic acid and 5 mM acetone formate, and Solvent B was made up of acetone formate with 0.1% formic acid and 5 mM ammonium formate. Linear gradient elution was done with 10-90% B (0-8 min) in 3 min at 10% B in 0-1 min, and re-equilibrated once again in 4 min, followed by another injection. The electrospray ionisation temperature was fixed at 500 °C, and the nebulising collision gas was nitrogen (99% pure). To achieve mass fragmentation, a collision energy of 35 eV was used, and a full-scan rate of 4000 Da/s was employed in both positive and negative modes in MS/MS. The AB SCIEX Analyst 1.5.2 and MS Processor software of Advanced Chemistry Development, Inc. (ACD/Labs, Ontario, Canada) was used to process, analyse, and interpret data. Metabolite searches were performed in the ACD and METLIN databases as previously described (Smith *et al*., 2005).

RESULTS

Bacterial Isolation and Primary Antimicrobial Activity Screening

Primary antimicrobial screening revealed that a number of bacterial isolates inhibited the growth of at least one of the clinical pathogens, *Enterococcus faecium, Klebsiella pneumoniae*, and *Acinetobacter baumannii* (Table 1). Sewage system and refuse dumps isolates had moderate inhibitory potential, especially against *E. faecium* and *A. baumannii*. Some isolates also showed selective inhibition of *K. pneumoniae*.

Bacterial identification

Partial 16S rRNA gene sequence (750bp) analysis showed that most bacterial isolates belonged to the genus Bacillus. The identified species included *Bacillus cereus*, *Bacillus paralicheniformis, Bacillus safensis*, and *Bacillus altitudinis*. Other isolates were identified as *Brevibacillus reuszeri* and *Enterobacter cloacae*. The partial 16S rRNA gene amplicons obtained from the isolates are shown in Figure 1. Sequence similarity analysis using the NCBI BLAST database revealed identities ranging from 92.12% to 100% with reference sequences in public databases. A summary of the identified bacterial species is presented in Table 1.

Antibacterial Activity of Ethyl Acetate Extracts

The antibacterial activity of ethyl acetate extracts from the bacterial isolates is summarised in Table 2, with representative inhibition zones shown in Figure 2. Among the extracts tested*, Bacillus altitudinis* (WP2) showed the greatest antibacterial activity. *Enterococcus faecium, Acinetobacter baumannii*, and *Klebsiella pneumoniae* were inhibited by the WP2 extract to 24 mm, 20 mm, and 16 mm, respectively. Conversely, extracts from the other isolates had minimal or no antimicrobial activity against the pathogens tested.

LC-MS/MS Profiling of Bioactive Metabolites

The chemical profile of the ethyl acetate extract from isolate WP2 is shown in Figure 3. Untargeted metabolomic analysis, combined with database matching, revealed several putative metabolites with reported biological activities. Among the predicted compounds were celastramycin A, cefditoren pivoxil, meclocycline, sericetin, and ascorbyl palmitate. The

detected metabolites, together with their molecular masses, chemical formulas, and reported biological functions, are summarised in Table 3.

Table 1: Preliminary screening of active isolates against pathogens and identification

Site of Cockroach	Bacterial Isolates	Closest species	Similarity %	*E. faecium*	*K. pneumoniae*	*A. baumannii*
Sewage systems	SS1	*Bacillus cereus* (AE016877)	92.59	+	-	+
	SS1	*Brevibacillus reuszeri* (LGIQ01000014)	99.86	+	-	+
	SS3	*Bacillus paralicheniformis* (LBMN01000156)	92.12	-	+	+
Refuse dumps	RD1	*Bacillus safensis* (ASJD01000027) 100	100	+	-	+
	RD2	*Enterobacter cloacae* (Z96079)	100	+	-	+
Waste disposal	WP1	*Bacillus safensis* (ASJD01000027) 99.86	99.86	+	+	+
	WP2	*Bacillus altitudinis* (ASJC01000029)	99.43	-	++	++

Keys: Negative (-), Positive (+), Very Positive (++)

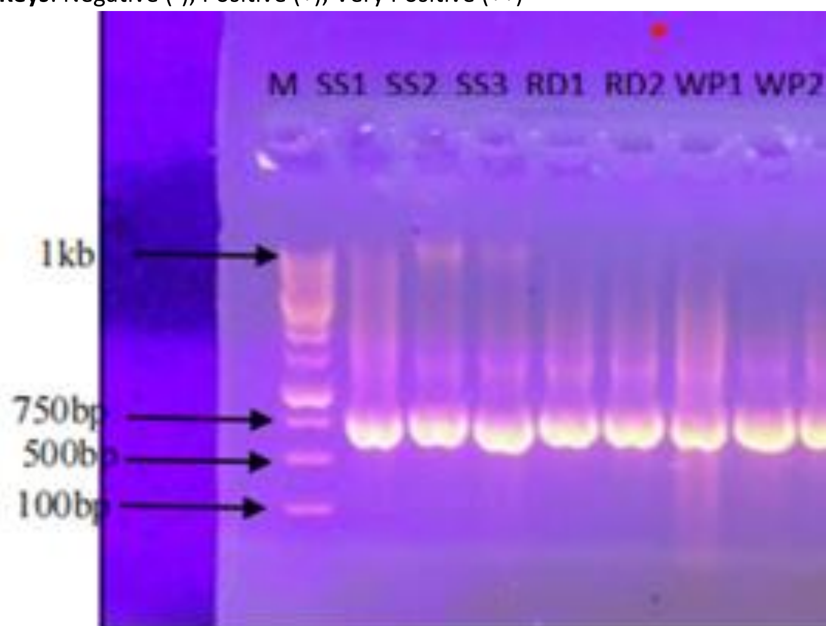

Fig.1: Gel electropherogram of bacterial isolates 16S rRNA gen

Table 2: Mean zone of inhibition of isolates' ethyl acetate extract against pathogens.

Bacterial Isolates	Zone of inhibition (mm)		
	E. faecium	***K. pneumoniae***	***A. baumannii***
SS1	-	-	-
SS2	5±0.03	-	-
SS3	-	-	-
RD1	-	-	-
RD2	-	-	-
WP1	-	-	-
WP2	24±0.05	16±0.01	20±0.02

Values are mean with standard deviation (±)

Fig.2: Zone of inhibition of isolates ethyl acetate extract against pathogens (a. *E. faeceium*, b. *A. baumannii*, c. *K. pneumoniae*)

Table 3: WP2 bacterial Isolate secondary metabolites profile

Predicted Compound	ESI Mode	m/z	Metlin ID	Chemical formula	Biological functions	References
9-oxo-11R,15R-dihydroxy-16,16-dimethyl-5Z,13E-prostadienoic acid	+	178.9420	36136	$C_{22}H_{36}O_5$	Anti-Ulcer agent	(Banerjee *et al*., 1979)
9α,15S-dihydroxy-11-oxothromba- 5Z,13E-dien-1-oic acid	+	179.9640	36259	$C_{20}H_{32}O_6$	Main plasma metabolites of TXB2 as a biomarker in patients with diabetes and arterial diseases	(Lopez *et al*., 2014)
(3-chloro-5-hexyl-2,6- dihydroxyphenyl) (4,5-dichloro-1H-pyrrol-3-yl)-methanone (Celastramycin A)	+	182.9850	45553	$C_{17}H_{18}Cl_3NO_3$	Antibacterial isolated from endophytic bacteria.	(Pullen *et al*., 2002)
Cefditoren pivoxil	+	197.0500	43981	$C_{25}H_{28}N_6O_7S_3$	Antibacterial agent	(Wellington & Curran, 2004)
Meclocycline	+	235.0150	43238	$C_{22}H_{21}ClN_2O_8$	A tetracycline antibiotic	(Feng *et al*., 2010)
Axitinib	-	249.1210	85189	$C_{22}H_{18}N_4OS$	An anti-cancer agent	(Bracarda *et al*., 2014)
(2S,3S)-2-(3-(4-aminobutoxy)-4 nitrobenzamido)-3-hydroxybutanoic acid	-	253.0810	65102	$C_{15}H_{21}N_3O_7$	RNase inhibitor	(Samanta *et al*., 2011)
Ascorbyl Palmitate	-	255.2350	73561	$C_{22}H_{38}O_7$	An antioxidant agent	(Let *et al*., 2007)
(2-hydroxy-1-pentyl-1H-indol-3-yl) (naphthalen-1-yl)-methanone	-	256.0780	64904	$C_{24}H_{23}NO_2$	An anti-nociception	(Aung *et al*., 2000)
Sericetin	-	259.0800	44599	$C_{25}H_{24}O_5$	An anti-viral agent	(Patil *et al*., 1993)

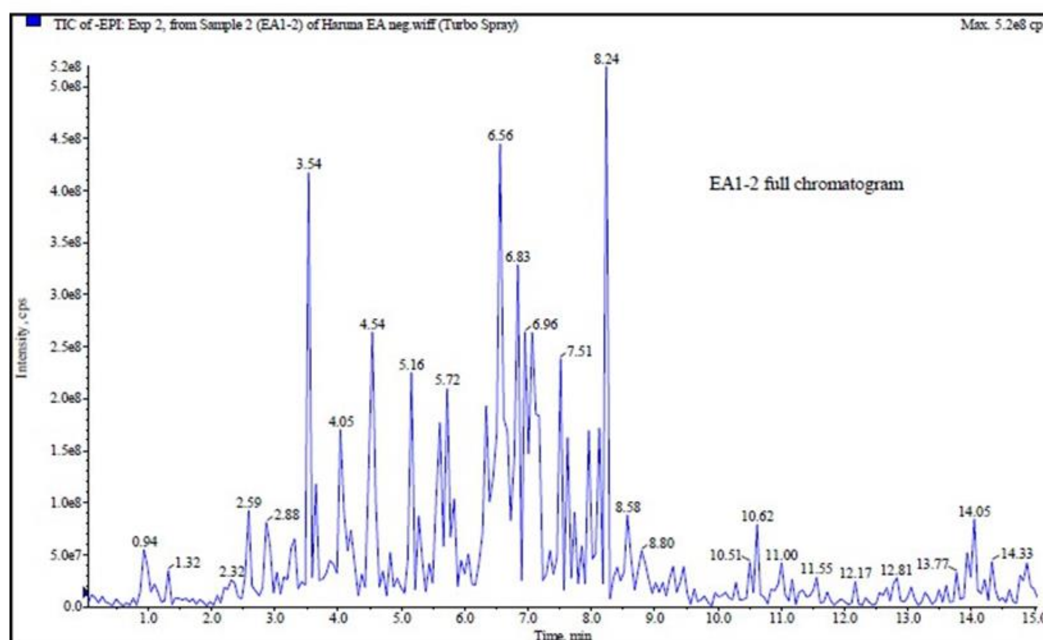

Fig.3: LC-MS/MS chromatogram of the WP2 ethyl-acetate extract

DISCUSSION

The current research shows that cockroaches in a highly polluted environment harbour a wide range of bacterial communities capable of producing antimicrobial compounds. Isolation of bacteria adapted to environments with high microbial competition was made possible by sampling sewage systems, refuse dumps, and waste disposal sites. These ecological conditions tend to favour microorganisms that produce antimicrobial metabolites that inhibit the competing microorganisms.

Molecular identification showed that most isolates belonged to the genus *Bacillus*, which is known to produce structurally diverse secondary metabolites. This genus is known to produce antibiotics, bacteriocins and lipopeptides, which help microbes compete and enhance their ecological fitness. It has already been documented that insect-associated *Bacillus* species commonly produce antimicrobial substances that safeguard the bacteria and their insect hosts against infectious microorganisms (Chen *et al*., 2024).

The main antimicrobial screening test showed that some of these isolates had an inhibitory activity against clinically significant pathogens. The most outstanding activity was, however, recorded in the isolate of WP2, which was *Bacillus altitudinis*. This isolate produces high levels of extracellular antimicrobial compounds, as evidenced by the ethyl acetate extract showing a significant inhibitory zone against all tested pathogens.

It is also notable that the observed activity against Gram-positive (*E. faecium*) and Gram-negative bacteria (*K. pneumoniae* and *A. baumannii*) is particularly remarkable, as these organisms are known to be significant opportunistic pathogens associated with hospital-acquired infections and growing antimicrobial resistance. The antimicrobial activity of metabolites may be broad-spectrum, as a single bacterial isolate can inhibit several pathogens.

Additional details on the chemical composition of the WP2 extract were revealed by LC-MS/MS-based metabolomic profiling. The chromatographic run showed several peaks, indicating the presence of secondary metabolites as predicted, several of which are known to have pharmacological activity. Some of the identified compounds include celastramycin A and meclocycline, which have been reported previously to possess antibacterial activity. Celastramycin A is a microbial source of antimicrobial agents, but meclocycline is an antimicrobial agent, an antibiotic, which is a tetracycline antibiotic that inhibits bacterial protein synthesis (Pullen *et al*., 2002; Feng *et al*., 2010). The presence of these compounds supports the antimicrobial effects observed in the bioassays.

The detection of several metabolites with diverse biological activities also supports the biosynthetic capacity of insect-related bacteria. Insects are also increasingly recognised as hosts of microorganisms with sophisticated biosynthetic gene clusters that produce a broad spectrum

of secondary metabolites. Genomics and metabolomics studies have also revealed that insect microbiomes harbour a host of untapped metabolic pathways that could serve as a useful source of new antimicrobial substances (Turner *et al*., 2024).

Despite the promising results of this research, it has several limitations that should be considered. Antimicrobial activity in this study was assessed using crude extracts; purified compounds were not used. Although preliminary evidence of bioactivity has been obtained from crude extracts, additional purification and structural characterisation of the compounds using technologies such as nuclear magnetic resonance (NMR) spectroscopy would be necessary to confirm the identity and biological activity of the identified metabolites.

CONCLUSION

Cockroaches in polluted environments have a high number of bacteria with antimicrobial potential. Among the isolates examined, *Bacillus altitudinis* (WP2) showed the greatest antibacterial activity against various clinically significant pathogens and produced a broad-spectrum bioactive extract associated with a range of metabolites. These results highlight insect-associated bacteria as potentially promising but under-explored sources of antimicrobial compounds, and justify future research into their biosynthetic capabilities for the discovery of antimicrobial drugs. More in-depth characterisation of the active metabolites, along with their biosynthetic pathways, will be significant for advancing their pharmaceutical applicability in the context of antimicrobial resistance.

Acknowledgement: We are grateful to the Tertiary Education Trust Fund (TETFUND) for funding this research under the Institutional Basic Research (IBR) grant, 2023.

Conflicts of interest statement: The authors declare no conflicts of interest.

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