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Acinetobacter Species Strain IrC1: A Copper-Biosorbing, Antibiotic-Resistant Environmental Isolate

(Spesies *Acinetobacter* Strain IrC1: Pencilan Persekitaran Rintang Antibiotik, Biopenjerapan Kuprum)

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ABSTRACT

Copper and antibiotic co-contamination presents environmental challenges, requiring sustainable remediation strategies. Copper bioremediation using environmentally resilient bacteria, such as *Acinetobacter* species, offers a promising approach. This study evaluated *Acinetobacter* sp. strain IrC1, isolated from the copper-contaminated Rungkut River (Surabaya, Indonesia), for its copper resistance and biosorption potential under antibiotic stress. Susceptibility to 16 antibiotics was assessed using the Kirby-Bauer disk-diffusion method. Minimum inhibitory and minimum bactericidal concentrations (MIC and MBC, respectively) were subsequently determined for ceftazidime, ciprofloxacin, and erythromycin. These experiments were conducted with 4 mM $CuSO_4$ or none. Copper biosorption was further tested at 4 mM $CuSO_4$ in the presence of selected antibiotics. Whole-genome sequencing was conducted to characterize the isolate and to identify resistance determinants. Exposure to 4 mM $CuSO_4$ reduced the strain's susceptibility to several antibiotics, as indicated by decreased median of inhibition zone diameters: piperacillin–tazobactam (22 to 19 mm), ceftazidime (19 to 15.5 mm), ciprofloxacin (25 to 19 mm), and erythromycin (16 to 12 mm). In subsequent MIC assays, the strain exhibited resistance to erythromycin under copper exposure (4 to 8 mg/L). However, its MBC remained unchanged. At 4 mM $CuSO_4$, ceftazidime and ciprofloxacin slightly decreased copper uptake, whereas erythromycin modestly increased biosorption (7.46 - 15.29%). However, these changes were not statistically significant ($p > 0.05$). Genomic analysis identified the strain as *Acinetobacter pittii* strain IrC1. Its copper resistance was associated with efflux pump systems, while antibiotic resistance involved both efflux mechanisms and antibiotic-inactivating enzymes. Most resistance genes were not located near insertion sequences, suggesting a low potential for horizontal gene transfer. These findings suggest that *A. pittii* IrC1 may have a potential to be used in copper bioremediation in environments contaminated by certain antibiotics.

Keywords: *Acinetobacter pittii*; antibiotics; biosorption; copper; multi resistance

ABSTRAK

Pencemaran bersama oleh kuprum dan antibiotik menimbulkan cabaran alam sekitar yang memerlukan strategi pemulihan mampan. Bioremediasi kuprum menggunakan bakteria yang tahan terhadap persekitaran, seperti spesies *Acinetobacter*, merupakan pendekatan yang berpotensi. Kajian ini menilai *Acinetobacter* sp. strain IrC1, yang dipencilkan dari Sungai Rungkut (Surabaya, Indonesia) yang tercemar kuprum, dari segi ketahanan terhadap kuprum dan potensi biopenjerapan di bawah tekanan antibiotik. Kerentanan terhadap 16 jenis antibiotik dinilai menggunakan kaedah resapan cakera Kirby–Bauer. Kepekatan perencatan minimum (MIC) dan kepekatan bakterisid minimum (MBC) kemudiannya ditentukan bagi ceftazidime, ciprofloxacin dan eritromisin dengan atau tanpa kehadiran 4 mM $CuSO_4$. Biopenjerapan kuprum turut diuji pada 4 mM $CuSO_4$ dalam kehadiran antibiotik terpilih. Penjujukan genom keseluruhan dijalankan untuk mencirikan pencilan dan mengenal pasti penentu rintangan. Pendedahan kepada 4 mM $CuSO_4$ mengurangkan kerentanan strain terhadap beberapa antibiotik, seperti ditunjukkan oleh penurunan diameter zon perencatan median: piperacillin–tazobactam (22 kepada 19 mm), ceftazidime (19 kepada 15.5 mm), ciprofloxacin (25 kepada 19 mm) dan eritromisin (16 kepada 12 mm). Dalam ujian MIC seterusnya, strain menunjukkan rintangan terhadap eritromisin di bawah pendedahan kuprum (4 kepada 8 mg/L), walaupun MBCnya kekal tidak berubah. Pada 4 mM $CuSO_4$, ceftazidime dan ciprofloxacin mengurangkan sedikit pengambilan kuprum, manakala eritromisin meningkatkan biopenjerapan secara sederhana (7.46 - 15.29%), namun perubahan ini tidak signifikan secara statistik ($p > 0.05$). Analisis genom mengenal pasti strain ini sebagai *Acinetobacter pittii* IrC1. Rintangan terhadap kuprum dikaitkan dengan sistem pam efluks, manakala rintangan antibiotik melibatkan

kedua-dua mekanisme efluks dan enzim penyahaktifan antibiotik. Kebanyakan gen kerintangan tidak terletak berhampiran jujukan sisipan, menunjukkan potensi rendah untuk pemindahan gen secara mendatar. Keputusan ini mencadangkan bahawa *A. pittii* IrC1 berpotensi digunakan dalam bioremediasi kuprum di persekitaran yang tercemar dengan antibiotik tertentu.

Kata kunci: *Acinetobacter pittii*; antibiotik; biopenjerapan; kuprum; rintang pelbagai

INTRODUCTION

Copper is one of the most widely used heavy metal elements in the pharmaceutical, electronics, mining, and construction industries. At low concentrations, copper serves as an essential element for cellular function. However, it becomes toxic at higher levels (Nur Hafizah et al. 2022). Excessive concentrations of copper could also accumulate in the environment and persist for long periods, posing serious risks to human health and ecosystem sustainability. Excessive copper accumulation in human beings has been associated with several diseases, including cancer, liver damage, and neurological disorders (Malekirad, Hassani & Abdollahi 2021).

Due to the increasing usage of copper across various industries, environmental copper pollution has expectedly risen. Several regions in Indonesia have been reported to be polluted with copper, with concentrations exceeding the limits set by the World Health Organization. Specifically, the maximum limits for copper concentration in drinking water and the environment are 2 and 0.001 mg/L, respectively. According to Dini et al. (2024), copper levels in three estuaries in Cirebon (i.e., Kesenden, Sukalila, and Kalijaga) had ranged from 0.008 to 0.011 mg/L. Badariah et al. (2023) had also reported that copper concentrations in the Batanghari River in Jambi ranged between 0.0241 and 0.075 mg/L. This indicates a necessity to have an effective technique(s) in reducing the copper pollutants in the environment.

Various conventional methods, such as precipitation, reverse osmosis, membrane filtration, ion exchange, and adsorption are commonly applied to treat heavy metal waste, including copper. However, those methods face several limitations, including high energy requirements, lengthy processing times, and the generation of toxic sludge. Certain techniques also involve the usage of large quantities of chemical reagents, which may cause secondary contamination. The effectiveness of those methods can be influenced by several factors, such as copper concentration and environmental conditions (Liu et al. 2023; Nur Hafizah et al. 2022). Hence, those methods are considered sub-optimal for copper remediation, necessitating the development of more effective alternatives.

Microbe-based bioremediation, such as *Acinetobacter* or *Pseudomonas* species, could offer a more efficient solution since it requires less energy, and this method is more cost-effective and environmentally friendly (Kugler et al. 2022). The presence of copper in contaminated environments can drive microorganisms living in those environments to develop resistance mechanisms that enable them to adapt and survive. Microorganisms capable of thriving in high-copper environments can accumulate copper in their cytoplasm or membrane fractions, making them suitable candidates for bioremediation agents (Irawati et al. 2023).

Besides copper, antibiotics are also common environmental contaminants. Antibiotics are widely used to combat bacterial infections in both humans and animals. However, environmental contamination arises from excessive usage, with antibiotics frequently detected in wastewater from anthropogenic activities, such as pharmaceutical production, domestic sewage, and livestock farming (Mutuku, Gazdag & Melegh 2022). The combined presence of copper and antibiotics in the environment can drive natural selection, leading to the emergence of bacterial strains resistant to both contaminants.

The presence of copper and ciprofloxacin in the environment had induced *Escherichia coli* K12 resistance towards other antibiotics, such as chloramphenicol, erythromycin, and tetracycline (Li et al. 2021). We recently had reported a similar finding on *Pseudomonas aeruginosa* PaD2, exhibiting resistance to copper as well as cefoxitin, erythromycin, and tetracycline (Jo et al. 2025). This suggests a correlation between copper and antibiotic resistance mechanisms. Multi-resistant bacterial strains generally possess enhanced adaptability and survival in contaminated environments, increasing the risk of spreading antibiotic-resistance genes that threaten human health (Haque et al. 2024). Nevertheless, bacteria with such abilities can also be exploited as bioremediation agents to mitigate copper and antibiotic contamination (Murray et al. 2024).

Despite increasing evidence of copper-induced co-selection for antibiotic resistance, it remains unclear whether bacterial isolates from copper-contaminated environments can sustain or enhance copper biosorption under antibiotic stress. Furthermore, although multi-resistance may enhance bacterial survival in co-contaminated environments, the genomic context of these resistance determinants, whether intrinsically encoded within the chromosome or plasmid, has not been thoroughly evaluated in candidate bioremediation strains. Understanding this distinction is crucial, as the environmental application of multi-resistant bacteria requires not only functional stability but also minimal risk of disseminating resistance genes.

Recently, the *Acinetobacter* species strain IrC1, isolated from the Rungkut River in Surabaya has been identified as another potential copper bioremediation agent. This strain could withstand up to 8 mM $CuSO_4$ and accumulated as much as 292.93 mg/g dry cell weight of copper (Irawati et al. 2023). *Acinetobacter* spp. are known to exhibit intrinsic

resistance to various classes of antibiotics (Reygaert 2018). This research was therefore conducted to measure the ability of *Acinetobacter* sp. IrC1 to tolerate copper and antibiotics, as well as its ability to absorb copper in the presence of antibiotics. Multi-resistance properties of *Acinetobacter* sp. IrC1 against antibiotics and copper were identified through both physiological and molecular approaches to evaluate its potential as a bioremediation agent.

MATERIALS AND METHODS

BACTERIAL MAINTENANCE

The bacterial strain used in this study was *Acinetobacter* species strain IrC1, isolated at the Biology Laboratory of Biology Education Department in Universitas Pelita Harapan, Indonesia. The isolate was obtained from activated sludge in an industrial wastewater treatment plant in Rungkut River, Surabaya, Indonesia (Irawati et al. 2012). The isolate was initially cultured in salt-based broth composed of (per liter) K_2HPO_4 1.5 g, KH_2PO_4 0.5 g, $(NH_4)_2SO_4$ 0.5 g, and $Mg_2SO_4.7H_2O$ 0.2 g, supplemented with the designated concentration of copper sulfate. Currently, the isolate was sub-cultured every two weeks onto fresh Luria–Bertani agar (HiMedia, India) supplemented with 4 mM $CuSO_4$ and stored at 4 °C (Irawati et al. 2023).

ANTIBIOTIC DISK-DIFFUSION ASSAY

Acinetobacter sp. IrC1 was grown in Mueller-Hinton broth (HiMedia, India) for approximately 4 h at 37 °C under shaking conditions at 150 rpm for aeration using a shaker incubator (Biobase, China). The bacterial turbidity used for the antibiotic disk-diffusion assay was between 0.3 and 0.8 (OD_{600}). The assay was performed on Mueller-Hinton agar (Oxoid, United Kingdom) under two conditions: (i) without $CuSO_4$ supplementation and (ii) with the addition of 4 mM $CuSO_4$. A total volume of 100 µL of bacterial culture was subsequently inoculated onto each medium using the swab method (Webber, Wallace & Burnham 2022). Sixteen antibiotic discs (Liofilchem, Italy) were used, i.e., gentamicin (10 µg), rifampicin (5 µg), piperacillin-tazobactam (110 µg), ceftazidime (30 µg), sulfonamide (300 µg), vancomycin (30 µg), clindamycin (2 µg), erythromycin (15 µg), mupirocin (5 µg), ampicillin (10 µg), oxacillin (5 µg), chloramphenicol (30 µg), lefamulin (30 µg), bacitracin (10 µg), ciprofloxacin (5 µg), and tetracycline (30 µg). Each antibiotic disc was aseptically placed on the surface of agar. The agar plates were subsequently incubated for 16-18 h at 37 °C. The diameters of the clear zones were measured and interpreted according to standard guidelines, such as CLSI M100 (2024) and EUCAST clinical breakpoint tables v14.0 (2024) (Khan, Siddiqui & Park 2019).

BROTH MICRODILUTION ASSAY

Acinetobacter sp. IrC1 was grown on Mueller-Hinton broth for 4 h at 37 °C under shaking conditions at 150 rpm using a shake incubator (Biobase, China). The cell density used for broth microdilution assay to determine minimum inhibitory concentration (MIC) was 1×10^8 cfu/mL, which was measured using a spectrophotometer at OD_{600} (Barnes et al. 2023). The assay was performed under two conditions: (i) without the addition of $CuSO_4$ and (ii) with the addition of 4 mM $CuSO_4$. The 4 mM concentration was chosen based on previous findings indicating bacterial survival at concentrations up to 8 mM, representing a sub-inhibitory copper stress level (Irawati et al. 2023). The antibiotics and respective concentrations were selected based on results of the disk-diffusion assay that displayed increased resistance, i.e., ceftazidime (1 g), erythromycin (500 mg), and ciprofloxacin (500 mg).

Antibiotic stock solutions were prepared by dissolving antibiotics in sterile distilled water to a concentration of 5,120 mg/L and were subsequently two-fold diluted to obtain the desired concentrations. In a 96-well plate, 100 µL of each antibiotic concentration was dispensed into the wells, followed by the addition of 100 µL (1×10^8 cfu/mL) of the prepared bacterial culture. A positive control contained 100 µL of bacterial culture and 100 µL of sterile broth without any antibiotic. A negative control contained 100 µL of each antibiotic concentration mixed with 100 µL of sterile broth. The plates were incubated for 18 h at 37 °C. Bacterial growth in each well was assessed visually, corroborated spectrophotometrically using a microplate reader at OD_{600}. The MIC for each antibiotic was interpreted according to the CLSI M100 (2024) clinical breakpoint tables (Barnes et al. 2023). The minimum bactericidal concentration (MBC) for tested antibiotics was subsequently assessed. Briefly, 25 µL of each incubated well was streaked onto Mueller-Hinton agar, without the addition of antibiotics and $CuSO_4$. The bacterial culture was incubated for 24 h at 37 °C, after which the MBC was determined (Parvekar et al. 2020).

COPPER BIOSORPTION ASSAY

Acinetobacter sp. IrC1 was cultured in Luria-Bertani broth (HiMedia, India) and incubated in a shaking incubator at 37 °C with agitation at 150 rpm until reaching a turbidity of 0.8 (OD_{600}). The assay was conducted in Luria-Bertani broth supplemented by either 3 mM or 4 mM $CuSO_4$, concentrations chosen based on previous tolerance data, as they represent sub-inhibitory copper stress (Irawati et al. 2023). The assay was conducted in the presence of ceftazidime (2 mg/L), ciprofloxacin (0.0625 mg/L), and erythromycin (4 mg/L), which were selected based on results of broth microdilution assays. A medium without antibiotics was used as the control. Additionally, an abiotic control (lacking bacterial inoculation) was included for each treatment condition and served as the analytical blank. The *Acinetobacter* sp. IrC1 cultures were inoculated into

each medium and incubated in a shaking incubator at 37 °C with agitation at 150 rpm for 48 h. Following incubation, the samples were centrifuged at 10,000 ×g for 10 min. The resulting supernatant was treated with HNO_3 to stabilize the sample and ensure that copper remained in a soluble form suitable for detection.

Copper concentration was subsequently quantified using the Atomic Absorption Spectroscopy (AAS) method by PT. BMT Asia Indonesia (Bogor, Indonesia). The AAS method quantifies copper by measuring the absorption of light at a specific wavelength, which is proportional to the element's concentration (Chengappa et al. 2025). Copper biosorption efficiency was calculated as: Removal (%) = $[(C_0 - C_e) / C_0] \times 100$, where C_0 and C_e represent the initial and treated copper concentrations, respectively.

STATISTICAL ANALYSIS

Results of copper biosorption were analyzed using the Scheirer–Ray–Hare test in RStudio (https://github.com/rstudio). This non-parametric test was selected as an alternative to two-way ANOVA, as the data did not meet the assumptions required for parametric analysis. The data were visualized using GraphPad Prism version 10.6.1 (GraphPad Software, Boston, MA, USA).

WHOLE-GENOME SEQUENCING AND BIOINFORMATIC ANALYSES

Genomic DNA of *Acinetobacter* sp. IrC1 was sequenced by PT. Genetika Science Indonesia (Tangerang, Indonesia) using the platform of Oxford Nanopore Technology (Oxford Nanopore, United Kingdom). The sequences were processed through filtering with Filtlong (https://github.com/rrwick/Filtlong) and transformed using Samtools (https://github.com/samtools/samtools). The sequence's read quality was evaluated using NanoPlot (https://github.com/wdecoster/NanoPlot), and *de novo* assembly was performed with Flye (https://github.com/fenderglass/Flye). The assembled sequence was polished three times with Medaka (https://github.com/nanoporetech/medaka) and four times with Racon (https://github.com/isovic/Racon). The sequence was subsequently mapped using Minimap2 (https://github.com/lh3/minimap2), and the assembly quality was assessed with QUAST (https://github.com/ablab/quast). The FASTA output was annotated using Prokka (https://github.com/tseemann/prokka).

Taxonomic assignment and genome completeness evaluation were performed using DFAST_QC (https://github.com/nigyta/dfast_qc). The annotated output was visualized with Proksee (https://proksee.ca/). The phylogenetic tree was constructed using TYGS (https://tygs.dsmz.de/) and visualized with iTOL (Interactive Tree of Life) (https://itol.embl.de/). Antibiotic resistance genes were screened using ABRicate (https://github.com/tseemann/abricate) with the Comprehensive Antibiotic Resistance Database (CARD) (https://card.mcmaster.ca/). Copper resistance genes were screened using BLASTp, based on the BacMet database (i.e., the antibacterial biocide and metal resistance genes database) on the Galaxy platform (https://usegalaxy.org/). Plasmid was detected using PlasmidHunter (http://hts.iit.edu/galaxy) and confirmed using NCBI BLASTn (https://blast.ncbi.nlm.nih.gov/Blast.cgi), while insertion sequence was detected using ISFinder (https://isfinder.biotoul.fr/). The complete chromosomal sequence of *Acinetobacter* sp. IrC1 had been submitted to the GenBank (#CP185978.1). Its plasmid sequence had been submitted to GenBank as well (#CP185979.1).

RESULTS AND DISCUSSION

The antibiotic resistance profile of *Acinetobacter* sp. IrC1 was assessed by disk-diffusion with 16 types of antibiotics in the presence of 4 mM $CuSO_4$ or none. Table 1 shows that *Acinetobacter* sp. IrC1 was resistant to many antibiotics, except piperacillin-tazobactam, ceftazidime, sulfonamide, ciprofloxacin, tetracycline, and bacitracin. The results reinforced the status of *Acinetobacter* sp. as a multidrug-resistant bacterium due to its high resistance levels against various classes of antibiotics (da Silva et al. 2023). *Acinetobacter* sp. has been reported to possess various antibiotic resistance mechanisms, i.e., biofilm formation, efflux pumps, enzymatic degradation of antibiotics, reduced membrane permeability, and modification of antibiotic target sites (Scoffone et al. 2025). Of note, the addition of 4 mM $CuSO_4$ did not significantly change many of its antibiotic resistance profiles. Interestingly, rifampicin was more effective in suppressing the bacterial growth in the presence of copper. The addition of copper was linked to the formation of reactive oxygen species, membrane damage, and thiol group binding (Cvijan, Korać Jačić & Bajčetić 2023; Verma & Kaushik 2020), which could potentially increase the effectiveness of rifampicin in this study. In contrast, certain antibiotics, such as erythromycin and ceftazidime, appeared to be weaker in suppressing the bacterial growth in the presence of copper. It was probable that exposure to copper could upregulate certain resistance mechanisms (efflux pump), which facilitated an increased resistance to erythromycin and ceftazidime as well (Bazzi et al. 2020; Chauhan et al. 2024; Murray et al. 2024).

The MICs of three antibiotics with less antibacterial effectiveness in the presence of copper (i.e., ceftazidime, ciprofloxacin, and erythromycin) were assessed. While the concentrations ranged from 64 to 0.125 mg/L for ceftazidime and erythromycin, the concentration of ciprofloxacin ranged from 8 to 0.015625 mg/L in this assay. *Acinetobacter* sp. IrC1 exhibited an increased resistance to ceftazidime and ciprofloxacin in the presence of 4 mM $CuSO_4$ (Table 2). However, the observed increases in MIC were relatively small. Thus, *Acinetobacter* sp. IrC1 remained classified as susceptible to these antibiotics according to the CLSI M100 (2024) breakpoints. In contrast, *Acinetobacter* sp. IrC1 displayed clear resistance to erythromycin in the presence of copper (Table 2).

Notably, Cu^{2+} ions may promote gene transfer among bacteria, further facilitating the dissemination of antibiotic resistance phenotypes in the environment. This effect is

likely associated with copper-induced stress, which can generate reactive oxygen species that damage bacterial DNA and activate the SOS response. The activation of this pathway is thought to be mediated by proteins, such as RecA, potentially facilitating the mobilization of mobile genetic elements that carry antibiotic resistance genes in bacteria (Folvarska et al. 2024; Raro, Poirel & Nordmann 2023). This suggested that exposure to copper could induce a multi-resistance mechanism towards several antibiotics.

The MBC was subsequently assessed for ceftazidime, ciprofloxacin, and erythromycin. This was performed by streaking samples from the abovementioned MIC experiments onto the surface of Mueller-Hinton agar without supplementation of antibiotic and copper. As depicted in Table 2, *Acinetobacter* sp. IrC1 was still able to grow on the concentrations exceeding its MIC, indicating that its MBC was not affected by the presence of copper. This was not surprising, as *Acinetobacter* sp. was known to have two types of survival strategies: (i) dormancy that reduces metabolic activity to increase resistance and (ii) a boom and bust model that uses dead cells as nutrients for living cells. Notably, both mechanisms may operate concurrently (Bravo et al. 2019), thereby increasing the organism's likelihood of survival despite exposures to heavy metals and antibiotics.

To investigate the potential of *Acinetobacter* sp. IrC1 for bioremediating copper, we performed the biosorption assay in the presence of selected antibiotics, i.e., ceftazidime (2 mg/L), ciprofloxacin (0.0625 mg/L), and erythromycin (4 mg/L). The assay tested two relatively low concentrations of copper, i.e., 3 and 4 mM $CuSO_4$ (sub-inhibitory copper stress concentrations), hence allowing subsequent challenge with antibiotics. The AAS method was performed to measure the remaining copper ions within each medium. *Acinetobacter* sp. IrC1 had a relatively modest ability to absorb copper from the environment (Figure 1). In treatment with 3 mM $CuSO_4$, the bacterial isolate exhibited a similar level of copper biosorption, with or without antibiotics. In treatment with 4 mM $CuSO_4$, the presence of ceftazidime or ciprofloxacin slightly reduced the bacterial ability to absorb copper. Intriguingly, the presence of erythromycin modestly increased the average copper biosorption level (7.46% to 15.29%). This suggested that the presence of erythromycin and 4 mM $CuSO_4$ as double stressors might stimulate the bacterial strain to absorb more copper from the environment. However, the statistical analysis indicated that the differences were insignificant ($p > 0.05$).

Bioinformatic analyses were subsequently performed to corroborate the abovementioned findings. The whole-

TABLE 1. Antibiotic resistance profiles of *Acinetobacter* sp. IrC1

Antibiotic Class	Antibiotic	Concentration	No $CuSO_4$		4 mM $CuSO_4$	
			Median (min–max)	R/I/S	Median (min–max)	R/I/S
Aminoglycoside	Gentamicin[a]	10 µg	14 (14–14)	I	14 (14–14)	I
Ansamycin	Rifampicin[c]	5 µg	16 (15–17)	R	21 (20–21)	S
β-lactam/β-lactamase inhibitors	Piperacillin-Tazobactam[a]	100/10 µg	22 (21–22)	S	19 (19–20)	I
Cephalosporin	Ceftazidime[a]	30 µg	19 (16–20)	S	15.5 (13–17)	I
Fluoroquinolone	Ciprofloxacin[a]	5 µg	25 (24–26)	S	19 (16–19)	I
Folate antagonist	Sulphonamides[c]	300 µg	21 (21–22)	S	21 (21–22)	S
Glycopeptide	Vancomycin[d]	30 µg	7 (7–7)	R	6 (6–6)	R
Lincosamide	Clindamycin[c]	2 µg	8 (8–8)	R	6 (6–6)	R
Macrolide	Erythromycin[c]	15 µg	16 (16–17)	I	12 (10–13)	R
Monocarboxylic acid	Mupirocin[c]	5 µg	6 (6–6)	R	10 (9–11)	R
Penicillin	Ampicillin[b]	10 µg	11 (11–12)	R	11 (11–12)	R
Penicillinase-resistant penicillin	Oxacillin[c]	5 µg	6 (6–6)	R	6 (6–6)	R
Phenicols	Chloramphenicol[c]	30 µg	16 (15–16)	I	14 (12–15)	I
Pleuromutilin	Lefamulin[f]	30 µg	6 (6–6)	R	6 (6–6)	R
Polypeptide	Bacitracin[e]	10 IU	14 (14–15)	S	16 (15–17)	S
Tetracycline	Tetracycline[a]	30 µg	25 (23–25)	S	24 (24–25)	S

R: Resistant; I: Intermediate; S: Susceptible. Inhibition zone diameters (in millimeters) were reported as the median of three technical replicates. For the selected antibiotics (ceftazidime, ciprofloxacin, and erythromycin), medians were calculated from three independent biological replicates, each measured in triplicates. [a]Based on CLSI M100 (2024) for *Acinetobacter* spp. [b]Based CLSI M100 (2024) for *Acinetobacter* spp. using breakpoints from the same antibiotic class. [c]Based on CLSI M100 (2024) for *Staphylococcus* spp. [d]Based on CLSI M100 (2024) for *Enterococcus* spp. [e]Based on the testing standard for *Vibrio* sp. in Amatul-Samahah et al. (2020). [f]Based on EUCAST Clinical Breakpoints v14.0 (2024) for *Staphylococcus* spp.

TABLE 2. Minimum inhibitory concentrations and minimum bactericidal concentrations of *Acinetobacter* sp. IrC1

Antibiotic Class	Antibiotic	No $CuSO_4$			4 mM $CuSO_4$		
		MIC (mg/L)	MBC (mg/L)	R/I/S	MIC (mg/L)	MBC (mg/L)	R/I/S
Cephems	Ceftazidime[a]	2	> 64	S	4	> 64	S
Fluoroquinolone	Ciprofloxacin[a]	0.0625	> 8	S	0.125	> 8	S
Macrolides	Erythromycin[b]	4	> 64	I	8	> 64	R

R: Resistant; I: Intermediate; S: Susceptible. Minimum inhibitory concentration (MIC) values were reported as the median of three independent biological replicates, each measured in technical triplicates. [a]Based on CLSI M100 (2024) for *Acinetobacter* spp. [b]Based on CLSI M100 (2024) for *Staphylococcus* spp. Minimum bactericidal concentration (MBC) values were also reported as the median of three biological replicates, each measured in technical triplicates

genome length of *Acinetobacter* sp. IrC1 (accession number CP185978.1) was 3,945,444 base pairs, with a guanine-cytosine (GC) content of 39.05% (Table 3). A total of 3,646 coding sequence genes were identified, with 2,799 and 847 proteins categorized as functional proteins and hypothetical proteins or with unknown functions, respectively (Figure 2). The genome size and GC content of this bacterial strain were similar to those of another *Acinetobacter* spp. For example, the genome length of *Acinetobacter pittii* BHS4 was approximately 3.9 million base pairs with a GC content of 38.97% (Saunders et al. 2021). Of note, the genome of *Acinetobacter* sp. IrC1 had an Average Nucleotide Identity (ANI) value of 97.04% as compared to *A. pittii* CIP 70.29, strongly suggesting that the genome belonged to *A. pittii* because an ANI value of ≥95% towards a reference genome was routinely used as the threshold for species-level classification (Aldeguer-Riquelme et al. 2024). Furthermore, the phylogenetic tree shown in Figure 3 demonstrated that the genome was closely related to *A. pittii* CIP 70.29, suggesting that *Acinetobacter* sp. IrC1 was a strain of *A. pittii*.

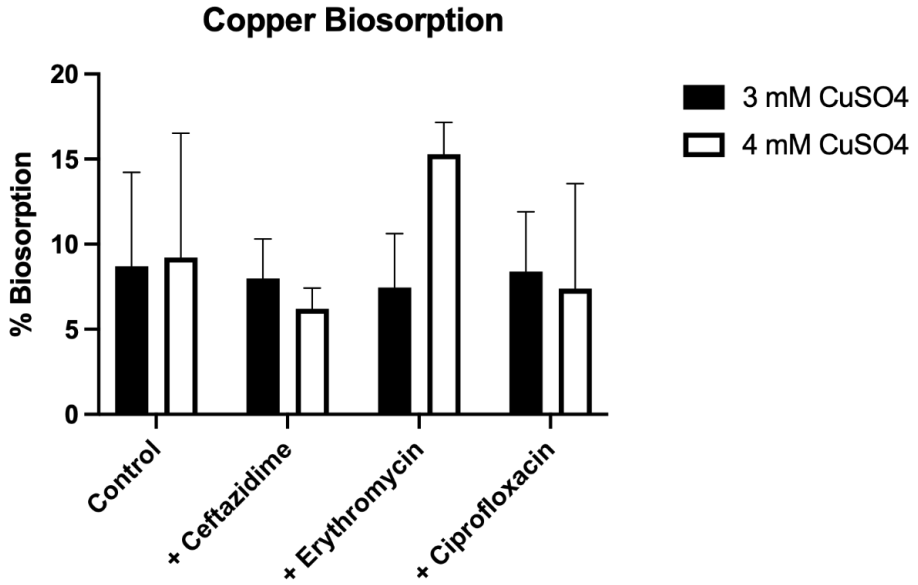
Subsequent bioinformatic analyses were conducted to identify antibiotic and copper resistance genes in the genome of *A. pittii* strain IrC1. The antibiotic resistance genes were selected based on percent identity of ≥90% and query coverage of ≥90% to ensure high-confidence detection (Anjum, Zankari & Hasman 2017). The antibiotic resistance genes presented in Table 4 were also selected based on the tested antibiotics.

Based on the identified genes, *A. pittii* IrC1 appeared to rely on the efflux pump mechanism to resist the suppressive effect of several antibiotics. The efflux pump mechanism could reduce the concentration of antibiotics within the bacteria by discharging antibiotics from the cell. Most identified efflux pumps were from the Resistance-Nodulation-Division (RND) family. The RND-type pumps, e.g., *adeI*, *adeJ*, and *adeK*, could mediate resistance to several antibiotics, including chloramphenicol, macrolides, and rifampicin (Zhu et al. 2025). In addition, the small multidrug resistance-type pump, e.g., *abeS*, had been reported to be responsible for the resistance to macrolides, such as erythromycin (Lin et al. 2017).

The copper resistance genes were selected based on a percent identity of ≥90% and query coverage of ≥90% to ensure high-confidence detection (Anjum, Zankari & Hasman 2017). However, as no gene was detected, the parameters were subsequently adjusted to ≥50% for percent identity, ≥80% for query coverage, and an E-value of $< 1\times10^{-10}$ to ensure an accurate gene prediction and reduce the risk of random matches. This approach enabled identification of similar copper resistance genes (Newell et al. 2013), as shown in Table 5.

Interestingly, the identified copper-resistance genes had various action mechanisms. While the *copA* gene was known to remove copper from the cytoplasm (hence reducing the risk of cellular damage due to copper toxicity), the *copR* gene was known to detect copper concentration and regulate the expression of *copA* (Mindlin et al. 2016). Although the function of the *copF* gene was not fully elucidated, it had been suggested to play a role in copper removal, in which this gene is activated in later stages of copper exposure (Alquethamy et al. 2019). Another identified copper-regulation mechanism involved a two-component system consisting of the *cusR* regulatory gene and the *cusA* gene, encoding an RND-type efflux pump. The *cusR* served as a response regulator, activating the expression of *cusA* in response to elevated concentrations of Cu^{+} ion. The synergistic activation of these genes regulated the *cus* operon, facilitating copper efflux through the RND type efflux pump system (Ramnarine et al. 2024).

Of note, genes involved in resistance to antibiotics and copper can be acquired through horizontal gene transfer (HGT), which is facilitated by mobile genetic elements, such as plasmid or insertion sequence (IS) (Han et al. 2025). Plasmid detection in the genome of *A. pittii* IrC1 was thus performed using PlasmidHunter (http://hts.iit.edu/galaxy), which predicted contig 2 of the isolated genome as a plasmid and was labelled as 'plra1'. This was further confirmed by NCBI BLASTn, which showed that contig 2 had a high similarity to plasmid unnamed1 from *Acinetobacter* sp. DUT-2, with 99.95% identity and 99% query coverage (data not shown). The plasmid size was also consistent with those commonly found in *Acinetobacter* spp., which generally ranged from 1.3 to 400 kb (Sánchez-Urtaza et al. 2024). Plasmid plra1 in *A. pittii* IrC1 contained 141 protein-coding genes that were hypothesized to contribute to the strain's ability to adapt to contaminated environments. However, as genes associated



The mean values from three independent assays were depicted in the figure. The error bars indicated standard deviations. All differences within or between groups were statistically not significant ($p > 0.05$)

FIGURE 1. Percentage of copper biosorption by *Acinetobacter* sp. strain IrC1

TABLE 3. Genome statistics of *Acinetobacter* sp. IrC1

Attribute	Value
Genome size (bp)	3,945,444
Contig	2
GC Content (%)	39.05%
Contig N50 (bp)	3,811,177
Contig L50	1
Plasmids	1
CDS	3,646
Total RNAs	91 (73 tRNA + 18 rRNA)

GC, guanine-cytosine; bp, base pair; N50, the sequence length of the shortest contig at 50% of the genome assembly; L50, the minimum number of contigs which the length sum makes up at least 50% of the genome assembly; CDS, coding sequence; tRNA, transfer RNA; rRNA, ribosomal RNA

with resistance to antibiotics and copper were not detected in this plasmid, the identified plasmid might not contribute to the acquisition or distribution of antibiotic and copper resistance genes. Plasmid pIra1 was likely to exert other roles, such as metabolic regulation or bacterial mobility (Thompson et al. 2023).

IS within the genome of *Acinetobacter pittii* IrC1 was subsequently identified using ISFinder (https://isfinder.biotoul.fr/) with a threshold parameter of E-value of 0.0001. A total of 7 IS elements were identified with significant matches, characterized by percentage identity ≥90% and an E-value of 0 (Table 6). IS enabled bacteria to acquire antibiotic and copper resistance genes from other bacteria, facilitating rapid adaptation (Consuegra et al. 2021). Analysis showed that the *adeK* and *adeJ* genes in the bacterial genome were located near to ISAba12, at distances of approximately 6,932 bp and 8,383 bp. Such relatively close distances (<10 kb) suggest the possible involvement of ISAba12 in the acquisition and mobilization of these genes through HGT (Hua et al. 2021). Nevertheless, no other antibiotic or copper resistance genes were found adjacent to IS elements in the genome. These findings indicated that most antibiotic and copper resistance genes in *A. pittii* IrC1 were less likely to be disseminated through HGT (Reygaert 2018), suggesting that this strain might be a reasonable candidate for copper bioremediation.

Acinetobacter spp. have been widely investigated for the bioremediation of environments contaminated with hydrocarbons, pesticides, and heavy metals (Muleshkova et al. 2025; OECD 2015). However, the environmental release of such microorganisms requires careful and context-specific risk assessment. Potential risks include

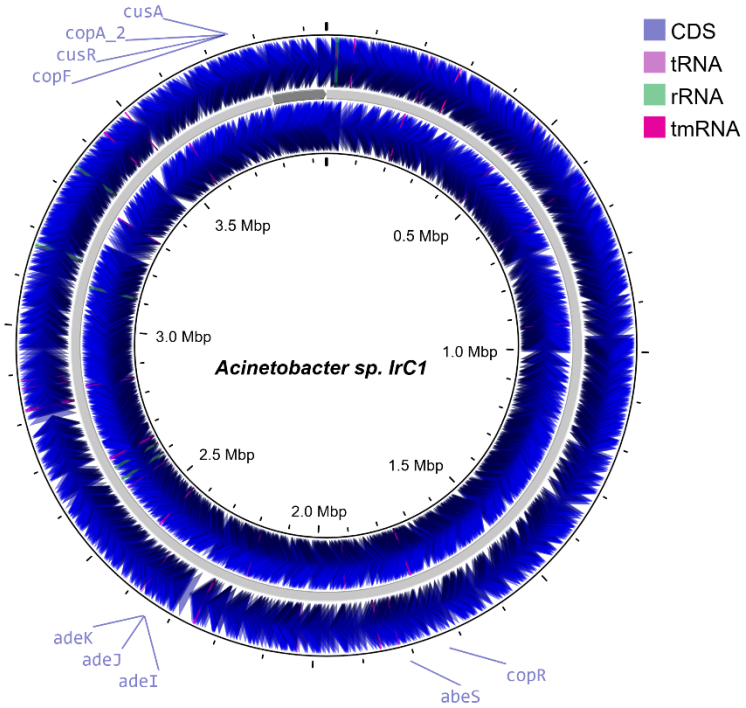


FIGURE 2. Visualization of the whole genome of *Acinetobacter* sp. IrC1. The visualization was performed with Proksee (https://proksee.ca/). The outermost blue circle displayed both forward and reverse coding sequences (CDS), with identified antibiotic and copper resistance genes labeled. Visualization of tRNAs (purple), rRNAs (green), and tmRNAs (pink) were added into the CDS. The innermost circle at the center represents the 3,945,444 base pairs (bp) that constituted its whole-genome length

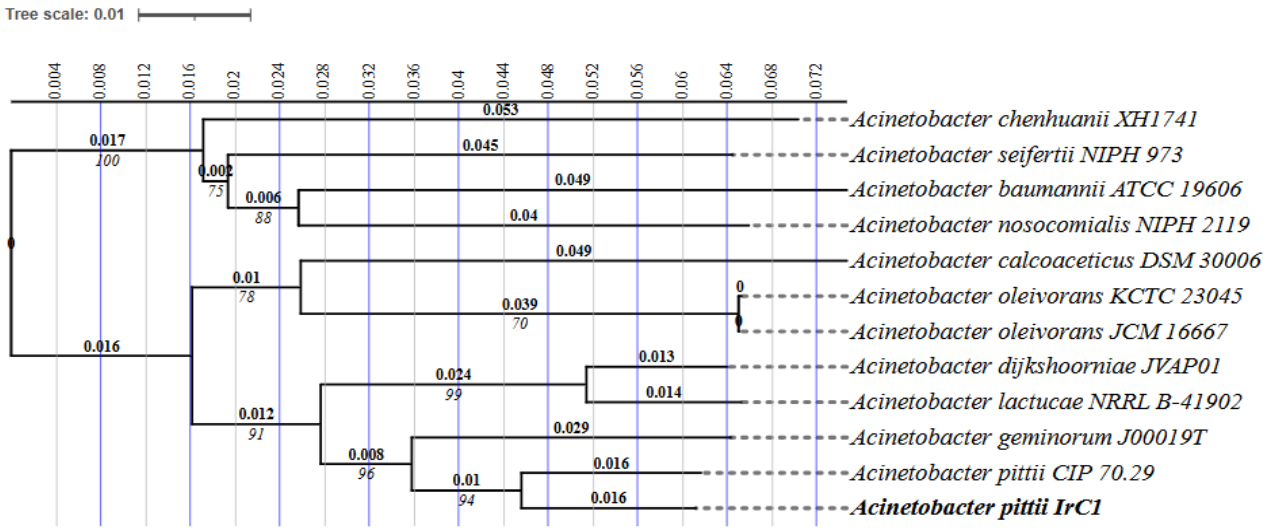


FIGURE 3. Phylogenetic tree of *Acinetobacter pittii* strain IrC1. TYGS (https://tygs.dsmz.de/) was used to compare *A. pittii* IrC1 to other *Acinetobacter* strains. iTOL (https://itol.embl.de/) was used to visualize the phylogenetic tree. The phylogenetic tree was generated using the MASH algorithm to compare the genome with other strains in the TYGS database. All pairwise intergenomic comparisons were conducted using GBDP and intergenomic distances were calculated with the d5 formula and trimming. The resulting intergenomic distances were used to construct the phylogenetic tree with the balanced minimum evolution method implemented in FASTME version 2.1.6.1, which included Subtree Pruning and Regrafting (SPR). Branch support was assessed with 100 pseudo-bootstrap replicates

TABLE 4. Antibiotic resistance genes in the genome of *Acinetobacter pittii* strain IrC1

Gene	Location (bp)	Family	Antibiotic resistance	Mechanism
abeS	1,808,288 – 1,808,617	*Small Multidrug Resistance*	*Aminocoumarin, macrolide*	Efflux Pump
adeI	2,342,612 – 2,343,859	*Resistance-Nodulation-Division*	*β-lactam, chloramphenicol, macrolide, rifampicin,* and *fusidic acid*	Efflux Pump
adeJ	2,343,872 – 2,347,048	*Resistance-Nodulation-Division*	*β-lactam, chloramphenicol, macrolide, rifampicin,* and *fusidic acid*	Efflux Pump
adeK	2,347,048 – 2,348,499	*Resistance-Nodulation-Division*	*β-lactam, chloramphenicol, macrolide, rifampicin,* and *fusidic acid*	Efflux Pump

TABLE 5. Copper resistance genes in the genome of *Acinetobacter pittii* IrC1

Gene	Location (bp)	AMR gene family	Heavy metal resistance	Resistance mechanism
copR	1,728,814 – 1,729,485	*MerR family*	Copper (Cu)	Activation of *copA* gene transcription
copF	3,740,563 – 3,742,902	*P-type ATPase*	Copper (Cu), Silver (Ag)	Cation efflux pump
copA	3,745,604 – 3,747,631	*P-type ATPase*	Copper (Cu)	Active efflux of Cu^{+} ions
cusR	3,744,346 – 3,745,029	*Two-component regulator*	Copper (Cu), Silver (Ag)	Regulation of the *cus* operon
cusA	3,750,587 – 3,752,644	*RND efflux*	Copper (Cu), Silver (Ag)	Monovalent metal ions efflux

TABLE 6. Insertion sequence (IS) in the genome of *Acinetobacter pittii* strain IrC1

Insertion Sequence	IS Family	Group	Origin	Location in genome (bp)
IS17	IS5	IS903	*Acinetobacter haemolyticus*	830,341 – 831,120
ISAba12	IS5	IS903	*Acinetobacter baumannii*	89,721 – 90,759; 111,301 – 112,339; 503,577 – 504,615; 738,271 – 739,309; 918,287 – 919,325; 1,514,888 – 1,515,926; 2,355,431 – 2,356,469
ISAba14	IS3	IS150	*Acinetobacter baumannii*	636,887 – 638,168; 1,216,184 – 1,217,465; 1,608,643 – 1,609,924; 2,150,600 – 2,151,881; 2,154,324 – 2,155,605; 3,799,882 – 3,801,163
ISAba26	IS256	-	*Acinetobacter baumannii*	1,016,308 – 1,017,625; 2,167,162 – 2,168,479
ISAba53	IS5	IS1031	*Acinetobacter baumannii*	3,457,792 – 3,458,830; 3,472,811 – 3,473,849
ISAba125	IS30	-	*Acinetobacter baumannii*	367,150 – 368,236; 729,383 – 730,469; 747,346 – 748,432; 776,268 – 777,354; 1,092,755 – 1,093,841; 1,238,895 – 1,239,981; 1,267,646 – 1,268,732; 1,749,180 – 1,750,266; 2,273,038 – 2,274,124; 2,380,515 – 2,381,601; 2,448,630 – 2,449,716; 2,718,575 – 2,719,661; 3,155,312 – 3,156,398; 3,250,952 – 3,252,038
ISAha2	IS5	IS903	*Acinetobacter haemolyticus*	1,467,313 – 1,468,352; 1,552,236 – 1,553,271

interactions with indigenous microbial communities, which may alter community structure, affect the persistence of the introduced strain, and facilitate horizontal transfer of resistance genes. These risks are highly dependent on site-specific environmental conditions, such as soil type, temperature, pH, oxygen availability, and nutrient status (Maglione et al. 2024; Sharma et al. 2022). Furthermore, the introduction of non-native microorganisms may disrupt local ecosystems, and microbial metabolic activity may generate secondary by-products requiring further management. Notably, in *A. pittii* IrC1, genes associated with copper and antibiotic resistance were not detected on plasmids, and many were not located adjacent to insertion sequence elements, suggesting a potentially lower risk of HGT. Nevertheless, the possibility of transferring resistance genes to indigenous microbes cannot be completely excluded, and HGT remains a key biosafety concern (OECD 2015). Several mitigation strategies have been proposed to mitigate the risk associated with environmental release, including the use of engineered control mechanisms (conditional survival systems or 'kill switches') and genome design approaches to reduce gene transfer potential (OECD 2015).

This study had several limitations. First, gene expression or transcriptomic analysis was not performed to complement the genomic data. Although phenotypic evidence of copper and antimicrobial resistance in *A. pittii* IrC1 was demonstrated, the absence of expression data impedes determination of the regulation and activation of the identified resistance genes. Second, virulence-associated features in its genome, including virulence genes, pathogenicity islands, and related risk factors, were not assessed. While these analyses were beyond the scope of the present study, they are important for evaluating the biosafety of *A. pittii* IrC1 for potential environmental applications. Third, for certain antibiotics lacking *Acinetobacter*-specific susceptibility breakpoints, reference criteria from other bacteria (particularly Gram-positive species) were used to interpret inhibition zone diameters. This approach may not accurately reflect true clinical susceptibility. Fourth, biosorption experiments were conducted at a single time point and under a fixed pH condition, limiting insight into biosorption kinetics and the influence of environmental parameters. Finally, copper removal was quantified using the AAS method, which measured total copper reduction in solution. This method cannot distinguish between surface adsorption and intracellular bioaccumulation. Elucidating these mechanisms would require additional techniques, such as electron microscopy, in future studies.

CONCLUSION

Whole-genome analysis indicated that the tested strain was *Acinetobacter pittii* strain IrC1. The strain demonstrated resistance to most classes of antibiotics, as well as tolerance to the exposure of 4 mM $CuSO_4$. Its resistance profile was supported by the detection of multiple resistance genes, including *adeI*, *adeJ*, and *adeK* for various antibiotics, as well as *copR*, *copA*, and *cusA* for copper. This bacterial strain exhibited a relatively modest copper biosorption capacity, suggesting that *A. pittii* IrC1 might have the potential to be used in copper bioremediation in environments contaminated by certain antibiotics. Further studies will be required to improve its capability to absorb copper as well as to ensure its safety profiles are utilized in the environment.

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REFERENCES

Aldeguer-Riquelme, B., Conrad, R.E., Antón, J., Rossello-Mora, R. & Konstantinidis, K.T. 2024. A natural ANI gap that can define intra-species units of bacteriophages and other viruses. *mBio* 15(8): e0153624.

Alquethamy, S.F., Khorvash, M., Pederick, V.G., Whittall, J.J., Paton, J.C., Paulsen, I.T., Hassan, K.A., McDevitt, C.A. & Eijkelkamp, B.A. 2019. The role of the *Copa* copper efflux system in *Acinetobacter baumannii* virulence. *International Journal of Molecular Sciences* 20(3): 575.

Amatul-Samahah, M.A., Muthukrishnan, S., Omar, W.H.H.W., Ikhsan, N.F.M. & Ina-Salwany, M.Y. 2020. *Vibrio* sp. associated with acute hepatopancreatic necrosis disease (AHPND) found in penaeid shrimp pond from east cost of Peninsular Malaysia. *Journal of Environmental Biology* 41(5(SI)): 1160-1170.

Anjum, M.F., Zankari, E. & Hasman, H. 2017. Molecular methods for detection of antimicrobial resistance. *Microbiology Spectrum* 5(6): ARBA-0011-2017.

Badariah, B., Sukmawati, N., Heraningsih, S.F., Rainiyati, R., Riduan, A. & Putri, R.D. 2023. Heavy metal contamination of Batanghari River, Jambi, Indonesia: Determination based on sediment enrichment factor value. *Journal of Degraded and Mining Lands Management* 10(4): 4761.

Barnes, V.L., Heithoff, D.M., Mahan, S.P., House, J.K. & Mahan, M.J. 2023. Antimicrobial susceptibility testing to evaluate minimum inhibitory concentration values of clinically relevant antibiotics. *STAR Protocols* 4(3): 102512.

Bazzi, W., Abou Fayad, A.G., Nasser, A., Haraoui, L-P., Dewachi, O., Abou-Sitta, G., Nguyen, V-K., Abara, A., Karah, N., Landecker, H., Knapp, C., McEvoy, M.M., Zaman, M.H., Higgins, P.G. & Matar, G.M. 2020. Heavy metal toxicity in armed conflicts potentiates AMR in *A. baumannii* by selecting for antibiotic and heavy metal co-resistance mechanisms. *Frontiers in Microbiology* 11: 68.

Bravo, Z., Orruño, M., Navascues, T., Ogayar, E., Ramos-Vivas, J., Kaberdin, V.R. & Arana, I. 2019. Analysis of *Acinetobacter baumannii* survival in liquid media and on solid matrices as well as effect of disinfectants. *Journal of Hospital Infection* 103(1): e42-e52.

Chauhan, R., Patel, H., Bhardwaj, B., Suryawanshi, V. & Rawat, S. 2024. Copper induced augmentation of antibiotic resistance in *Acinetobacter baumannii* MCC 3114. *BioMetals* 38(2): 485-504.

Chengappa, S.K., Rao, A., Holla, S.R., Shenoy, R., Pai, B.H.M., Jodalli, P. & Avinash, B.R. 2025. Analysis of copper, zinc, arsenic, and lead content of over-the-counter toothpastes from India: An *in vitro* study. *Scientific Reports* 15(1): 26535.

Clinical Laboratory Standards Institute (CLSI). 2024. *Performance Standards for Antimicrobial Susceptibility Testing (34th ed., CLSI supplement M100)*. https://clsi.org/about/news/ast-news-update-january-2024-practical-tips-for-using-newly-formatted-tables-1-in-clsi-m100-33rd-edition/

Consuegra, J., Gaffé, J., Lenski, R.E., Hindré, T., Barrick, J.E., Tenaillon, O. & Schneider, D. 2021. Insertion-sequence-mediated mutations both promote and constrain evolvability during a long-term experiment with bacteria. *Nature Communications* 12(1): 980.

Cvijan, B., Korać Jačić, J. & Bajčetić, M. 2023. The impact of copper ions on the activity of antibiotic drugs. *Molecules* 28(13): 5133.

da Silva, M.E., Gomes, M.A., Rodrigues, R.S., Lima, N.C., Carvalho, A.G., Taborda, R.L. & Matos, N.B. 2023. Multidrug-resistant *Acinetobacter* spp. from hospital intensive care units in Brazilian Amazon. *The Brazilian Journal of Infectious Diseases* 27(6): 103687.

Dini Hartiningsih, Skalalis Diana, Yuniarti MS, Mochamad Rudyansyah Ismail & Qurnia Wulan Sari. 2024. Water quality pollution indices to assess the heavy metal contamination: A case study of the estuarine waters in Cirebon City (West Java, Indonesia) pre- and post-care COVID-19. *Environmental and Sustainability Indicators* 21: 100318.

EUCAST. 2024. *Breakpoint Tables for Interpretation of MICs and Zone Diameters (Version 14.0)*. https://www.eucast.org/clinical_breakpoints/

Folvarska, V., Thomson, S.M., Lu, Z., Adelgren, M., Schmidt, A., Newton, R.J., Wang, Y. & McNamara, P.J. 2024. The effects of lead, copper, and iron corrosion products on antibiotic resistant bacteria and antibiotic resistance genes. *Environmental Science: Advances* 3(6): 808-818.

Han, B., Feng, C., Jiang, Y., Ye, C., Wei, Y., Liu, J. & Zeng, Z. 2025. Mobile genetic elements encoding antibiotic resistance genes and virulence genes in *Klebsiella pneumoniae*: Important pathways for the acquisition of virulence and resistance. *Frontiers in Microbiology* 16: 1529157.

Haque, M.A., Rogerson, L., Deb Nath, N., Haruna, S., Ahn, J., Johnston, T.V., Lin, C.S., Chong, L., Na, L., Jang, M.J. & Ku, S. 2024. Sustainable management and valorization of antibiotic waste. *Chemical Engineering Journal* 498: 155372.

Hua, X., Liang, Q., Deng, M., He, J., Wang, M., Hong, W., Wu, J., Lu, B., Leptihn, S., Yu, Y., & Chen, H. 2021. BACANT: A combination annotation server for bacterial DNA sequences to identify antibiotic resistance genes, integrons, and transposable elements. *Frontiers in Microbiology* 12: 649969.

Irawati, W., Yuwono, T., Pinontoan, R. & Lindarto, V. 2023. Optimising wastewater treatment: *Acinetobacter* sp. IrC1 as a potential multi-resistant bacterium for copper accumulation and dyes decolourisation. *Tropical Life Sciences Research* 34(3): 37-56.

Irawati, W., Yuwono, T., Soedarsono, J. & Hartiko, H. 2012. Molecular and physiological characterization of copper-resistant bacteria isolated from activated sludge in an industrial wastewater treatment plant in Rungkut-Surabaya, Indonesia. *Microbiology Indonesia* 6(3): 107-116.

Jo, J., Datta, D.A., Irawati, W. & Purnomo, J.S. 2025. Physiological and molecular characterization of copper and antibiotic resistance mechanisms in *Pseudomonas aeruginosa* strain PAD2. *Biodiversitas Journal of Biological Diversity* 26(4): 1526-1536.

Khan, Z.A., Siddiqui, M.F. & Park, S. 2019. Current and emerging methods of antibiotic susceptibility testing. *Diagnostics* 9(2): 49.

Kugler, A., Brigmon, R.L., Friedman, A., Coutelot, F.M., Polson, S.W., Seaman, J.C. & Simpson, W. 2022. Bioremediation of copper in sediments from a constructed wetland *ex situ* with the novel bacterium *Cupriavidus basilensis* SRS. *Scientific Reports* 12(1): 17615.

Li, J., Phulpoto, I.A., Zhang, G. & Yu, Z. 2021. Acceleration of emergence of *E. coli* antibiotic resistance in a simulated sublethal concentration of copper and tetracycline co-contaminated environment. *AMB Express* 11(1): 14.

Lin, M-F., Lin, Y-Y., Tu, C-C. & Lan, C-Y. 2017. Distribution of different efflux pump genes in clinical isolates of multidrug-resistant *Acinetobacter baumannii* and their correlation with antimicrobial resistance. *Journal of Microbiology, Immunology and Infection* 50(2): 224-231.

Liu, Y., Wang, H., Cui, Y. & Chen, N. 2023. Removal of copper ions from wastewater: A review. *International Journal of Environmental Research and Public Health* 20(5): 3885.

Maglione, G., Zinno, P., Tropea, A., Mussagy, C.U., Dufossé, L., Giuffrida, D. & Mondello, A. 2024. Microbes' role in environmental pollution and remediation: A bioeconomy focus approach. *AIMS Microbiology* 10(3): 723-755.

Malekirad, A.A., Hassani, S. & Abdollahi, M. 2021. Oxidative stress and copper smelter workers. In *Toxicology,* edited by Patel, V.B. & Preedy, V.R. Academic Press. pp. 119-126.

Mindlin, S., Petrenko, A., Kurakov, A., Beletsky, A., Mardanov, A. & Petrova, M. 2016. Resistance of permafrost and modern *Acinetobacter lwoffii* strains to heavy metals and arsenic revealed by genome analysis. *BioMed Research International* 2016: 3970831.

Muleshkova, T., Bazukyan, I., Papadimitriou, K., Gotcheva, V., Angelov, A. & Dimov, S.G. 2025. Exploring the multifaceted genus Acinetobacter: The facts, the concerns and the opportunities the dualistic genus Acinetobacter. *Journal of Microbiology and Biotechnology* 35: e2411043.

Murray, L.M., Hayes, A., Snape, J., Kasprzyk-Hordern, B., Gaze, W.H. & Murray, A.K. 2024. Co-selection for antibiotic resistance by environmental contaminants. *NPJ Antimicrobials and Resistance* 2(1): 9.

Mutuku, C., Gazdag, Z. & Melegh, S. 2022. Occurrence of antibiotics and bacterial resistance genes in wastewater: Resistance Mechanisms and antimicrobial resistance control approaches. *World Journal of Microbiology and Biotechnology* 38(9): 152.

Newell, P.D., Fricker, A.D., Roco, C.A., Chandrangsu, P. & Merkel, S.M. 2013. A small-group activity introducing the use and interpretation of BLAST. *Journal of Microbiology & Biology Education* 14(2): 238-243.

Nur Hafizah Ab Hamid, Muhamad Iqbal Hakim bin Mohd Tahir, Amreen Chowdhury, Abu Hassan Nordin, Anas Abdulqader Alshaikh, Muhammad Azwan Suid, Nurul 'Izzah Nazaruddin, Nurul Danisyah Nozaizeli, Shubham Sharma & Ahmad Ilyas Rushdan. 2022. The current state-of-art of copper removal from wastewater: A review. *Water* 14(19): 3086.

Organisation for Economic Co-operation and Development (OECD). 2015. *Biosafety and the Environmental Uses of Micro-Organisms: Conference Proceedings*. OECD Publishing. http://dx.doi.org/10.1787/9789264213562-en

Parvekar, P., Palaskar, J., Metgud, S., Maria, R. & Dutta, S. 2020. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of silver nanoparticles against *Staphylococcus aureus*. *Biomaterial Investigations in Dentistry* 7(1): 105-109.

Ramnarine, S.D., Ali, O., Jayaraman, J. & Ramsubhag, A. 2024. Early transcriptional changes of heavy metal resistance and multiple efflux genes in *Xanthomonas campestris* pv. *campestris* under copper and heavy metal ion stress. *BMC Microbiology* 24(1): 81.

Raro, O.H., Poirel, L. & Nordmann, P. 2023. Effect of zinc oxide and copper sulfate on antibiotic resistance plasmid transfer in *Escherichia coli*. *Microorganisms* 11(12): 2880.

Reygaert, C.W. 2018. An overview of the antimicrobial resistance mechanisms of bacteria. *AIMS Microbiology* 4(3): 482-501.

Sánchez-Urtaza, S., Ocampo-Sosa, A., Rodríguez-Grande, J., El-Kholy, M.A., Shawky, S.M., Alkorta, I. & Gallego, L. 2024. Plasmid content of Carbapenem resistant *Acinetobacter baumannii* isolates belonging to five international clones collected from hospitals of Alexandria, Egypt. *Frontiers in Cellular and Infection Microbiology* 13: 1332736.

Saunders, B.H., Lai, G.K.K., Griffin, S.D.J. & Leung, F.C.C. 2021. Complete genome sequence of *Acinetobacter pittii* BHS4, isolated from air-conditioning condensate in Hong Kong. *Microbiology Resource Announcements* 10(42): e00880-21.

Scoffone, V.C., Trespidi, G., Barbieri, G., Arshad, A., Israyilova, A. & Buroni, S. 2025. The evolution of antimicrobial resistance in *Acinetobacter baumannii* and new strategies to fight it. *Antibiotics* 14(1): 85.

Sharma, P., Parakh, S.K., Singh, S.P., Parra-Saldívar, R., Kim, S-H., Varjani, S. & Tong, Y.W. 2022. A critical review on microbes-based treatment strategies for mitigation of toxic pollutants. *Science of the Total Environment* 834: 155444.

Thompson, C.M., Hall, J.P., Chandra, G., Martins, C., Saalbach, G., Panturat, S., Bird, S.M., Ford, S., Little, R.H., Piazza, A., Harrison, E., Jackson, R.W., Brockhurst, M.A. & Malone, J.G. 2023. Plasmids manipulate bacterial behaviour through translational regulatory crosstalk. *PLOS Biology* 21(2): e3001988.

Verma, V., & Kaushik, D. 2020. Mupirocin mounted copper nanoparticle offered augmented drug delivery against resistant bacteria. *Indian Journal of Pharmaceutical Education and Research* 54(3): 637-646.

Webber, D.M., Wallace, M.A. & Burnham, C.-A.D. 2022. Stop waiting for tomorrow: Disk diffusion performed on early growth is an accurate method for antimicrobial susceptibility testing with reduced turnaround time. *Journal of Clinical Microbiology* 60(5): e03007-20.

Zhu, Y., Hind, C.K., Al-Adhami, T., Wand, M.E., Clifford, M., Sutton, J.M. & Rahman, K.M. 2025. C7-substituted quinolines as potent inhibitors of *AdeG* efflux pumps in *Acinetobacter baumannii*. *ACS Infectious Diseases* 11(3): 626-638.

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