

Emerging antibiotic resistance in *Bacillus* spp. Isolated from geothermal hot springs in Sri Lanka

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Introduction and Objectives: Antibiotic resistance in bacteria is a global health concern. Geothermal hot springs, with their elevated temperatures and mineral-rich conditions provide distinct ecological niches that can foster the survival and adaptation of antibiotic-resistant microorganisms. This study examines the emerging patterns of antibiotic resistance in *Bacillus* spp. isolates collected from six geothermal springs in Sri Lanka.

Methods: *Bacillus* spp. isolates from six geothermal springs, Mahapelessa (n=5), Mahaoya (n=8), Nelumwewa (n=12), Rathkiriya (n=1), Kinniyai (n=6), and Wahawa (n=3) were confirmed by real-time PCR with *Bacillus*-specific primers. Six biochemically verified isolates were sequenced for species-level identification, and the rest were validated through qPCR assays. Antibiotic sensitivity tests (ABST) were performed using the Kirby-Bauer disk diffusion method with 12 antibiotics: erythromycin, chloramphenicol, tetracycline, gentamicin, clindamycin, levofloxacin, ciprofloxacin, amikacin, ceftazidime, imipenem, cefepime, and meropenem per CLSI standards. Real-time PCR analysis identified resistance genes associated with macrolides (*ermC*, *ermD*, *ermK*), lincosamides (*ermD*, *D/K*), phenicols (*cat*), and fluoroquinolones (*gyrA*, *parC*).

Results: Antibiotic susceptibility testing showed variable resistance patterns across different drug classes. Among macrolides, resistance to erythromycin (4/5 isolates) and clindamycin (4/5) was highest in Mahapelessa. Most isolates from all hot springs exhibited intermediate resistance to tetracycline. Beta-lactam resistance was notable for ceftazidime in Kinniya (3/5) and Nelum Wewa (10/12). Meropenem resistance was observed in isolates from Nelum Wewa (1/12) and Kinniya (1/6), whereas isolates from other hot springs remained susceptible. Fluoroquinolones and aminoglycosides demonstrated high efficacy against most isolates, with only minor resistance observed in Mahapelessa. Figure 1 illustrates the prevalence of *cat*, *ermD/K*, *ermC*, *gyrA*, and *parC* among *Bacillus* isolates from geothermal springs.

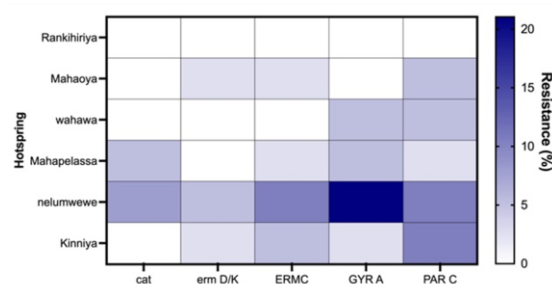


Figure 01: Heatmap showing the prevalence of selected antibiotic resistance genes

Conclusions: As indicated by ABST, the isolates were highly resistant to lincosamides and susceptible to fluoroquinolones. The isolates obtained from Mahapelessa, Nelumwewa, and Kinniya exhibited comparatively higher levels of antibiotic resistance than isolates from other sites. Isolates from Rathkiriya and Wahawa exhibited little to no resistance, likely reflecting minimal anthropogenic influence and limited exposure to pollutants or antibiotics, resulting in lower selective pressure for the development of resistance compared to isolates from the other sites. These findings highlight the interplay between environmental factors, human activity, and the distribution of antibiotic-resistant *Bacillus* isolates in geothermal ecosystems.

Keywords: Anthropogenic, Antibiotic resistance genes, *Bacillus* sp., Hot springs

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