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Genomic elucidation of antibiotic resistance genes in *Enterobacter* spp. isolated from livestock waste in Kandy district Sri Lanka using nanopore sequencing

LRLS Kumari[1], M Siriwardhana[1], HRN Jinadasa[3], WRP Wijesinghe[1,2]

Introduction and Objectives: Antibiotic resistance (ABR) is a major global health security issue. It is predicted that the current global death toll of 700,000 per annum will rise to 10 million by 2050 if vigorous actions are not taken. *Enterobacter* spp. are common nosocomial pathogens that acquire multi-drug resistance (MDR) in addition to their intrinsic β lactam resistance. The purpose of this study was to identify the antibiotic resistance genes from selected bacterial isolates from farms in the Kandy district using Oxford Nanopore sequencing.

Methods: Cow dung samples collected from fifteen farms were screened for the presence of β lactam resistance using a 96 well plate high throughput assay using 50 µg/ml of amoxicillin. Bacteria from wells that had growth on the initial 96 well assay plates were grown on nutrient agar with the same concentration of amoxicillin to obtain isolated colonies. Selected four colonies were cultured on nutrient broth and DNA was extracted using the QIAamp DNA mini kit. Bacterial genome and plasmid DNA were sequenced using the Oxford Nanopore® MinION Rapid Barcoding Sequencing kit (SQK-RBK004). The generated FastQ files from MinKNOW software were analysed using EPI2ME with WIMP and ARMA.

Results: Nanopore sequencing detected the taxonomic classification and resistance genes with the Phred quality score ≥ Q8. According to the WIMP analysis (threshold = 10000), one strain of ARB was identified as *Enterobacter cloacae* and the other three isolates as *Enterobacter hormaechei*. According to ARMA results, four strains showed 0.08%, 0.09%, 0.08% & 0.1% read-count as ABR genes with 98% accuracy. *E. cloacae* had efflux pumps (*CRP, H-NS*) for macrolides, penicillins, fluoroquinolones, tetracycline, cephamycin and cephalosporins; and target site alterations at aminoglycoside (*Ecol_16S_STR*) and peptide (*Ecol_16S_EDN* genes) binding sites. The three *E. hormaechei* isolates possessed altered aminoglycoside binding sites. Two of the *E. hormaechei* strains contained genes responsible for antibiotic inactivation (*ACT-25*), and antibiotic target replacement (*sul2*). The β lactam resistant determinants were present in chromosomal DNA while the rest of the resistant genes were located on plasmids

Conclusions: Selected four bacterial strains were identified as *E. cloacae* and *E. hormaechei* and they all showed genetic evidence of being multi-drug resistant (MDR). In general, the β lactam resistance related genes were present in chromosomal DNA while the rest of the resistant genes were located on plasmids.

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[1]*Department of Botany, Faculty of Science, University of Peradeniya, Sri Lanka*
[2]*Postgraduate Institute of Science, University of Peradeniya, Sri Lanka*
[3]*Department of Veterinary Pathology, Faculty of Veterinary Medicine & Animal Science, University of Peradeniya, Sri Lanka*

Address for correspondence: Dr. Priyanga Wijesinghe. Telephone: +94703335221

Email: priyangaw@sci.pdn.ac.lk https://orcid.org/0000-0002-7736-5038