**Supplementary tables**

Table 1. PCR master mix per tube and conditions for CTX-M, SHV and OXA

|  |  |
| --- | --- |
| **Reagent** | **Volume (µL)** |
| 10X ExTaq buffer | 5 |
| dNTP mixture | 4 |
| Template | 2 |
| Forward primer (10 μM) | 5 |
| Reverse primer (10 μM) | 5 |
| Nuclease-free water | 28.75 |
| TaKaRa ExTaq HS | 0.25 |
| **Total** | **50 µL** |

|  |  |  |
| --- | --- | --- |
| 98 °C | 2 min |  |
| 98 °C | 10 sec |  |
| 60.5 °C | 5 sec | x 25 cycles |
| 72 °C | 1 sec |  |
| 72 °C | 8 min |  |
| 4 °C | ∞ |  |

Table 2. PCR master mix per tube and conditions for TEM

|  |  |
| --- | --- |
| **Reagent** | **Volume (µL)** |
| 10X ExTaq buffer | 5 |
| dNTP mixture | 4 |
| Template | 2 |
| Forward primer (10 μM) | 5 |
| Reverse primer (10 μM) | 5 |
| Nuclease-free water | 28.75 |
| TaKaRa ExTaq HS | 0.25 |
| **Total** | **50 µL** |

|  |  |  |
| --- | --- | --- |
| 94 °C | 7 min |  |
| 94 °C | 30 sec |  |
| 57 °C | 30 sec | x 30 cycles |
|  72-°C | 30 sec |  |
| 72 °C | 5 min |  |
| 4 °C | ∞ |  |

Table 3: Primers used in this study

|  |  |  |
| --- | --- | --- |
| **NAME OF PRIMERS** | **TARGET GENE** | **SEQUENCE 5’-3’** |
| **TEM1F****TEM1R** | *bla*TEM | ATGAGTATTCAACATTTCCGCTGACAGTTACCAATGCTTA |
| **SHVF****SHVR** | *bla*SHV | GGTTATGCGTTATATTCGCCTTAGCGTTGCCAGTGCTC |
| **CTX-MA1****CTX-MA2** | *bla*CTX-M | \*SCSATGTGCAG≠YACCAGTAACCGC¥RATATGRTTGGTGGTG |

Note: \*S = G or C, ≠Y = C or T, ¥R = A or T.



Figure 1 Importation of antibiotics from 2015 to 2020



Figure 2 Antibiotic sales from March 2021 to February 2022



Figure 3:Overall AST for 16 *Enterobacteriaceae* strains



Figure 3 AST by Species



Figure 5: Map of Zambia showing the study areas