

APPENDIX A: EXTRACTION OF WHOLE-CELL BACTERIAL DNA.

Method:

1. Inoculate one selected colony in 10 ml liquid broth and incubate overnight at 37°C while shaking continuously.
2. Aliquot 1 ml into a sterile centrifuge tube and centrifuge at 3000 x g for 10 min.
3. Remove the supernatant and resuspend the precipitate in 1 ml distilled water.
4. Centrifuge at 3000 x g for 10 min and remove the supernatant.
5. Resuspend the pellet in 500 µl SET buffer (75 mM NaCl, 25 mM EDTA, 20 mM Tris pH 7.5).
6. Add 25 µl of a 20 % SDS solution and 1 µl of lysozyme (50 mg/ml).
7. Incubate the mixture for 1h at 37°C, then add 220 µl of NaCl (5M).
8. Add 700 µl chloroform/isoamylalcohol (24:1) and shake the tube vigorously.
9. Centrifuge at 5000 x g for 10 min, then remove the upper phase and transfer into a new tube.
10. Add 700 µl cold isopropanol (-20°C), then gently shake by inversion and let precipitate for 1h at -20°C.
11. Centrifuge at 5000 x g for 10 min, then remove the isopropanol, don't touch the pellet and add 800 µl 70% cold ethanol (-20°C).
12. Centrifuge at 5000 x g for 5 min, then remove the ethanol and add 800 µl cold 70% ethanol (-20°C) again.
13. Centrifuge at 5000 x g for 5 min, remove the ethanol, let dry and resuspend the visible (or not) pellet in 1ml TE buffer (10 mM Tris/HCl, 1mM EDTA, pH 7.4).
14. Store extracted DNA at 4°C until further analysis.

APPENDIX B: PUBLICATIONS FROM THIS THESIS.

1. **Weldhagen G.F., Poirel L. and Nordmann P.** 2003. Ambler class A extended-spectrum beta-lactamases in *Pseudomonas aeruginosa* – Novel developments and clinical impact. *Antimicrobial Agents and Chemotherapy*. **47**: 2385 - 2392.
2. **Weldhagen G. F.** 2004. Integrons and beta-lactamases – A novel perspective on resistance. *International Journal of Antimicrobial Agents* **23(6)**: 556 – 562.
3. **Weldhagen G. F.** 2004. Class A, extended –spectrum beta-lactamase production in *Pseudomonas aeruginosa* – Fact or Fiction? (Review article). *The Southern African Journal of Epidemiology and Infection*. **18(4)**: 77 – 84.
4. **Weldhagen G. F. and Prinsloo A.** 2004. Molecular detection of GES-2 extended spectrum beta-lactamase producing *Pseudomonas aeruginosa* in Pretoria, South Africa. *International Journal of Antimicrobial Agents*. **24(1)**: 35 – 38.
5. **Weldhagen G. F.** 2004. Sequence-selective recognition of beta-lactamase GES-2 by a competitive, peptide nucleic acid based, multiplex PCR assay. *Antimicrobial Agents and Chemotherapy*. **48(9)**: 3402 – 3406.
6. **Weldhagen G. F.** 2004. Rapid detection and sequence-specific discrimination of extended-spectrum beta-lactamase GES-2, from *Pseudomonas aeruginosa* with a real-time PCR assay. *Antimicrobial Agents and Chemotherapy*. **48(10)**: 4059 – 4062.