**SUPLEMENTARY MATERIALS FOR PAPER: Antibiotic Resistance Genes Occurrence in Wastewaters from Selected Pharmaceutical Facilities in Nigeria**

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**Table 1.** Description of sampled sites.

| Area | Sample codes | Site Location | Sample | Sample description |
|---|---|---|---|---|
| **Agbara Ogun State** | **WTPi** | $6^0$ 30′ N $3^0$ 5′ E | Untreated wastewater | Central untreated wastewater holding tank |
| | **WTPii** | | Treated water | Treatment tank |
| | **RWi** | $6^0$ 29′ N $3^0$ 6′ E | River water | Upstream of receiving water |
| | **RWii** | $6^0$ 29′ N $3^0$ 6′ E | River water | Downstream of receiving water |
| | **WWii1** | $6^0$ 30′ N $3^0$ 4′ E | Wastewater | Wastewater channel |
| | **WWii2** | $6^0$ 30′ N $3^0$ 4′ E | Wastewater | Wastewater holding tank |
| | **WWiv** | $6^0$ 30′ N $3^0$ 6′ E | Wastewater | Wastewater outlet |
| | **WWv** | $6^0$ 30′ N $3^0$ 4′ E | Wastewater | Stored wastewater |
| **Sango Ota Ogun State** | **WWi1** | $6^0$ 30′ N $3^0$ 5′ E | Wastewater | Holding tank |
| | **WWi2** | $6^0$ 30′ N $3^0$ 5′ E | Wastewater | Discharge point |
| | **WWvi** | $6^0$ 42′ N $3^0$ 13′ E | Wastewater | Outlet to the public drainage |
| **Table S1 (Cont'd.)** | | | | |
| **Ikeja-Lagos State** | **WWiii** | $7^0$ 39′ N $5^0$ 3′ E | Wastewater | Holding thank |
| | **WWvii** | $9^0$ 46′ N $5^0$ 56′ E | Wastewater | Discharge channel |
| | **WWviii** | $6^0$ 10′ N $5^0$ 18′ E | Wastewater | Holding tank |
| | **WWix** | - | Wastewater | Wastewater channel |
| **Oshodi-Lagos State** | **WWx** | - | Wastewater | Holding tank wastewater |

| | | | | |
|---|---|---|---|---|
| **Isolo-Lagos State** | **WWxi** | - | Wastewater | Holding tank |
| | **WWxii** | - | Wastewater | Discharge point |
| | **WWxiii** | - | Wastewater | Holding tank |
| | **WWxiv** | - | Wastewater | Holding tank |

**Table S2.** Primers and conditions used to amplify tetracycline resistance genes using the PCR techniques.

| Target Gene | Primer | Sequence (5′-3′) | Amplicon size (bp) | Annealing temp. ($^0$C) | Reference |
|---|---|---|---|---|---|
| *tet*(A) | TetA-F | GCTACATCCTGCTTGCCTTC | 210 | 55 | Ng *et al.*, 2001 |
| | TetA-R | CATAGATCGCCGTGAAGAGG | | | |
| tet(B) | TetB-F | TACGTGAATTTATTGCTTCGG | 206 | 55 | Ng et al., 2001 |
| | TetB-R | ATACAGCATCCAAAGCGCAC | | | |
| *tet*(C) | TetC-F | CTTGAGAGCCTTCAACCCAG | 418 | 55 | Ng *et al.*, 2001 |
| | TetC-R | ATGGTCGTCATCTACCTGCC | | | |
| *tet*(E) | TetE-F | AAACCACATCCTCCATACGC | 278 | 55 | Ng *et al.*, 2001 |
| | TetE-R | AAATAGGCCACAACCGTCAG | | | |
| *tet*(G) | TetG-F | GCTCGGTGGTATCTCTGCTC | 468 | 60 | Ng *et al.*, 2001 |
| | TetG-R | AGCAACAGAATCGGGAACAC | | | |
| *tet*(J) | TetJ-F | CGAAAACAGACTCGCCAATC | 184 | 61 | Aminov *et al.*, 2002 |
| | TetJ-R | TCCATAATGAGGTGGGGC | | | |
| *tet*(O) | TetO-F | AACTTAGGCATTCTGGCTCAC | 515 | 55 | Ng *et al.*, 2001 |
| | TetO-R | TCCCACTGTTCCATATCGTCA | | | |
| *tet*(X) | TetX-F | CAATAATTGGTGGTGGACCC | 468 | 55 | Ng *et al.*, 2001 |
| | TetX-R | TTCTTACCTTGGACATCCCG | | | |
| *tet*(M) | TetM-F | ACAGAAAGCTTATTATATAAC | 171 | 55 | Aminov *et al.*, 2001 |

| | | | | | |
|---|---|---|---|---|---|
| | TetM-R | TGGCGTGTCTATGATGTTCAC | | | |
| tet(T) | TetT-F | AAGGTTTATTATATAAAAGTG | 169 | 46 | Aminov *et al.*, 2001 |
| | TetT-R | AGGTGTATCTATGATATTTAC | | | |
| *tet*(W) | TetW-F | GAGAGCCTGCTATATGCCAGC | 168 | 64 | Aminov *et al.*, 2001 |
| | TetW-R | GGGCGTATCCACAATGTTAAC | | | |
| *tet*(Q) | TetQ-F | AGAATCTGCTGTTTGCCAGTG | 169 | 63 | Aminov, *et al.*, 2001 |
| | TetQ-R | CGGAGTGTCAATGATATTGCA | | | |
| *tet*(Z) | TetZ-F | CCTTCTCGACCAGGTCGG | 204 | 61 | Aminov *et al.*, 2002 |
| | TetZ-R | ACCCACAGCGTGTCCGTC | | | |
| *tet*(D) | TetD-F | GGAATATCTCCCGGAAGCGG | 187 | 68 | Aminov *et al.*, 2002 |
| | TetD-R | GGAATATCTCCCGGAAGCGG | | | |
| *tet*(BP) | TetBP-F | AAAACTTATTATATTATAGTG | 169 | 46 | Aminov *et al.*, 2001 |
| | TetBP-R | TGGAGTATCAATAATATTCAC | | | |
| *tet*(Y) | TetY-F | ATTTGTACCGGCAGAGCAAAC | 181 | 68 | Aminov *et al.*, 2002 |
| | TetY-R | GGCGCTGCCGCCATTATGC | | | |

**Table S3.** Primers and Conditions used to amplify Aminoglycosides and β-Lactam Resistance Genes using the PCR techniques.

| Target Gene | Sequence (5′-3′) | Amplicon size (bp) | Annealing temp. ($^0$C) | Reference |
|---|---|---|---|---|
| *aac*(3)-IV | TGCTGGTCCACAGCTCCTTC<br>CGGATGCAGGAAGATCAA | 653 | 56 | Heuer *et al.*, 2002 |
| *aac*(6′)-Ib(*aac*A4) | TGACCTTGCGATGCTCTATG<br>TTAGGCATCACTGCGTGTTC | 509 | 55 | Heuer *et al.*, 2002 |
| *aac*(3)-II | ACTGTGATGGGATACGCGTC<br>CTCCGTCAGCGTTTCAGCTA | 237 | 60 | Heuer *et al.*, 2002 |
| *aac*(3)-I | ACCTACTCCCAACATCAGCC<br>ATATAGATCTCACTACGCGC | 169 | 60 | Heuer *et al.*, 2002 |

| | | | | |
|---|---|---|---|---|
| *aac*(3)-III | GAAGTACGCAGAAGAGA<br>ACATGGCAAGCTCTAGGA | 491 | 58 | Heuer *et al*., 2002 |
| *ant*(3′′)-Ia (*aad*A) | GTGGATGGCGGCCTGAAGCC<br>AATGCCCAGTCGGCAGCG | 529 | 68 | Vakulenko *et al*., 2003 |
| *ant*(6)-I(*aad*E) | ACTGGCTTAATCAATTTGGG<br>GCCTTTCCGCCACCTCACCG | 577 | 58 | Vakulenko *et al*., 2003 |
| *ant*(2′′)-Ia (*aad*B) | ATGGACACAACGCAGGTCAC<br>TTAGGCCGCATATCGCGACC | 534 | 59 | Vakulenko *et al*., 2003 |
| *aph*(3′)-Ia (*aph*A1) | ATGGGCTCGCGATAATGTC<br>CTCACCGAGGCAGTTCCAT | 600 | 58 | Vakulenko *et al*., 2003 |
| *aph*(3′′)-I (*str*A) | CCTGGTGATAACGGCAATTC<br>CCAATCGCAGATAGAAGGC | 546 | 55 | Vakulenko *et al*., 2003 |
| *aph*(6)-Id (*str*B) | ATCGTCAAGGGATTGAAACC<br>GGATCGTAGAACATATTGGC | 509 | 56 | Vakulenko *et al*., 2003 |
| *bla*-TEM | ATAAAATTCTTGAAGACGAA A<br>GACAGTTACCAATGCTTAATC A | 1076 | 50 | Mabilat and Goussard, 1993 |
| *bla*-OXA | GTCTTTCGAGTACGGCATTA<br>ATTTTCTTAGCGGCAACTTAC | 720 | 55 | Vahaboglu *et al*., 1998 |

| | | | | |
|---|---|---|---|---|
| *bla*-IMP | CTACCGCAGCAGAGTCTTTG AACCAGTTTTGCCTTACCAT | 587 | 50 | Senda *et al*., 1996 |
| *bla*-CTX-M | TTTGCGATGTGCAGTACCAGT AA CGATATCGTTGGTGGTGCCAT A | 544 | 55 | Edelstein *et al*., 2003 |
| *bla*-NDM-1 | GTAAAACGACGGCCAG CAGGAAACAGCTATGAC | 1705 | 55 | Walsh *et al*., 2011 |

**Table S4.** Primers and Conditions used to amplify MLS, Sulfonamide and Chloramphenicol Resistance integron Genes using the PCR techniques.

| Target Gene | Sequence (5′-3′) | Amplicon size (bp) | Annealing temp. ($^0$C) | Reference |
|---|---|---|---|---|
| *erm*A | CCTGTCGGAATTGGTTTTTAG CGGTAAACCCCTCTGAGAATA | 452 | 52 | Chung *et al*., 1999 |
| *erm*B | GAAAAGGTACTCAACCAAATA AGTAACGGTACTTAAATTGTTTAC | 639 | 54 | Chung *et al*., 1999 |
| *erm*C | AGTAATGCCAATGAGCGTTTT GGTGTAATTTCGTAACTGCCA | 303 | 43 | Sutcliffe *et al*., 1996 |
| *ere*A | AACACCCTGAACCCAAGGGACG CTTCACATCCGGATTCGCTCGA | 466 | 56 | Sutcliffe *et al*., 1996 |
| *sul*I | ATCGCAATAGTTGGCGAAGT GCAAGGCGGAAACCCGCGCC | 798 | 55 | National Food Institute |
| *sul*II | GCGCTCAAGGCAGATGGCATT GCGTTTGATACCGGCACCCGT | 284 | 70 | National Food Institute, Denmark |
| *sul*III | GAGCAAGATTTTTGGAATCG CATCTGCAGCTAACCTAGGGCTTTGGA | 789 | 53 | National Food Institute, Demark |

| | | | | |
|---|---|---|---|---|
| *cat*A1 | CGCCTGATGAATGCTCATCCG<br>CCTGCCACTCATCGCAGTAC | 750 | 60 | National Food Institute, Denmark |
| *cml*A | TACTCGGATCCATGCTGGCC<br>TCCTCGAAGAGCGCCATTGG | 578 | 50 | National Food Institute, Denmark |
| *intI*1 | CCTCCCGCACGATGATC<br>TCCACGCATCGTCAGGC | 280 | 55 | Goldstein *et al.*, 2001 |

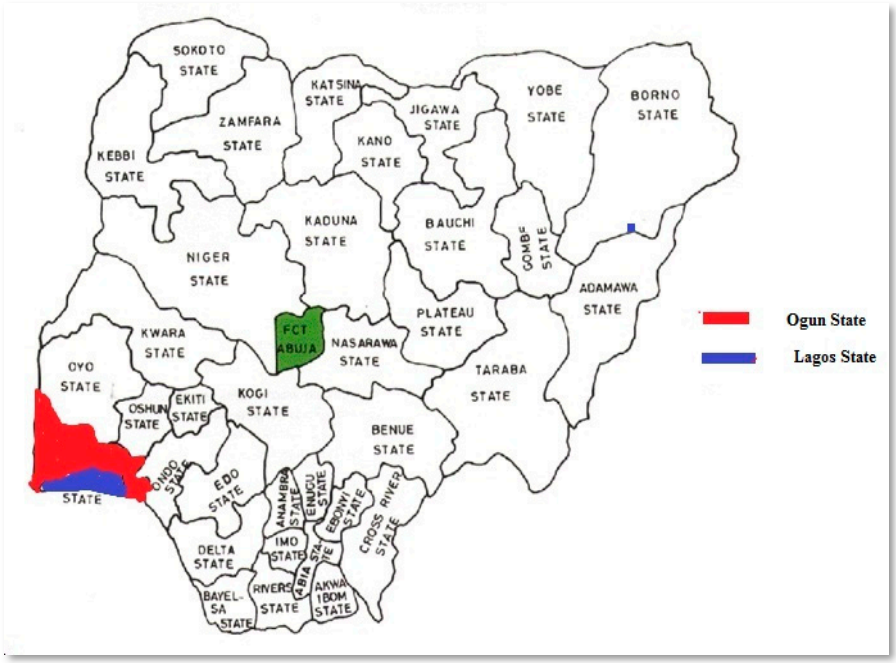


**Figure S1.** Map of Nigeria highlighting the study areas: Ogun and Lagos States.

**Figure S2.** A section of the map of Nigeria showing Agbara Industrial Area in Ogun State.

**Figure S3.** Cross-section of the wastewater treatment Plant located in Agbara Industrial Estate, in Ogun State, Nigeria.

## References


1. Ng, L.K.; Martin, I.; Alfa, M.; Mulvey, M. Multiplex PCR for the detection of tetracycline resistant genes. *Mol. Cel.l Probe*. **2001**, 15, 4, 209–215.
2. Aminov, R.I.; Garrigues-Jeanjean, N.; Mackie, R.I. Molecular ecology of tetracycline resistance: Development and validation of primers for detection of tetracycline resistance gene encoding ribosomal protection proteins. *Appl. Environ. Microbiol*. **2001**, 67, 1, 22–32.
3. Aminov, R.I.; Chee-Sanford, J.C.; Garrigues, N.; Teferedegne, B.; Krapac, I.J.; White, B.A.; Mackie, R.I. Development, validation, and application of PCR primers for detection of tetracycline efflux genes of gram-negative bacteria. *Appl. Environ. Microbiol*. **2002**, 68, 4, 1786–1793.
4. Heuer, H.; Krögerrecklenfort, E.; Wellington, E.M.H.; Egan, S.; van Elsas, J.D.; van Overbeek, L.; Collard, J.M., Guillaume, G.; Karagouni, A.D.; Nikolakopoulou, T.L.; Smalla, K.; Gentamicin resistance genes in environmental bacteria: prevalence and transfer. *FEMS Microbiol. Ecol*. **2002**, 42, 289–302.
5. Vakulenko, S.B.; Donabedian, S.M.; Voskresenskiy, A.M.; Zervos, M.J.; Lerner; S.A.; Chow, J.W. "Multiplex PCR for detection of aminoglycoside resistance genes in enterococci," *Antimicrob. Agents Chemother*. **2003**, 47, 4, 1423–1426.
6. Mabilat, C.; Goussard, S. PCR detection and identification of genes for extended-spectrum β-lactamases. In Diagnostic Molecular Microbiology: Principles and Applications. Persing, D.H.; Smith, T.F.; Tenover, F.C.; White, T. J. (eds). Washington, DC: *American Society for Microbiology*. **1993**, 553–563.
7. Vahaboglu, H.; Ozturk, R.; Akbal, H.; Saribas, S.; Tansel, O.; Kunkan, F.C. Practical approach for detection and identification of OXA-10-derived ceftazidime hydrolyzing extended-spectrum b-lactamases. *J. Clin. Microbiol.* **1998**, 36, 827–829.
8. Senda, K.; Arakawa, Y.; Ichiyama, S.; Nakashima, K.; Ito, H.; Ohsuka, S.; Shimokata, K.; Kato, N.; Ohta, M. PCR detection of metallo-β-lactamase gene (*bla*$_{IMP}$) in Gram-negative rods resistant to broad-spectrum β-lactams. *J. Clin. Microbiol.* **1996**, 34, 2909–2913.
9. Edelstein, M.; Pimkin, M.; Palagin, I.; Edelstein, I.; Stratchounski, L. Prevalence and molecular epidemiology of CTX-M extended-spectrum b-lactamase producing *Escherichia coli* and *Klebsiella pneumoniae* in Russian hospitals. *Antimicrob. Agents Chemother*. **2003**, 47, 3724–3732.
10. Walsh, T.R.; Weeks, J.; Livermore, D.M.; Toleman, M.A. Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study. *Lancet Infect Dis*. **2011**, 11, 355–62.
11. Chung, W.O.; Werckenthin, C.; Schwarz, S.; Roberts, M.C. Host range of the *erm*F rRNA methylase gene in bacteria of human and animal origin. *J. Antimicrob. Chemother.* **1999**, 43, 1, 5-14.

12. Sutcliffe, J.; Tait-Kamradt, A.; Wondrack, L. *Streptococcus pneumoniae* and *Streptococcus pyogenes* resistant to macrolides but sensitive to clindamycin: a common resistance pattern mediated by an efflux system. *Antimicrob. Agents Chemother.* **1996**. 40, 8, 1817-1824.
13. Goldstein, C.; Lee, M.D.; Sanchez, S.; Hudson, C.; Phillips, B.; Register, B.; Grady, M.; Liebert, C.; Summers, A.O.; White, D.G. Incidence of class 1 and 2 integrases in clinical and commensal bacteria from livestock, companion animals, and exotics. *Antimicrob. Agents Chemother*. **2001**, 45, 3, 723-726.