Supplementary Material

Supplementary Text File 1

Methodology of liquid chromatography–tandem mass spectrometry

Thawing of frozen water samples was done in a water bath. All subsequent steps were performed at +4°C. Water samples (100 µL) were rigorously mixed with 100 µL of deuterated internal standard solution followed by centrifugation at 11,000 rpm for 5 minutes. An aliquot of each sample was chromatographed on various analytical columns (Ascentis Express RP-Amide 4.6 x 50 mm 2.7 µm; Phenomenex Kinetex 2.5 µm 50 x 4.6 mm; Phenomenex Kinetex 2.5µm F5 50 x 4.6 mm; PLRP-S, 5 µ, 50 x 4.6 mm; SeQuant ZIC-HILIC 4.6 x 50 mm 5 µm; Symmetry® C18, 50 x 4.6 mm, 3.5 µm; YMC-Pack Phenyl, 50 x 40 mm, 3 µm), eluted with isochromatic or gradient solvent systems. Analytes and internal standards were monitored by LC-MS/MS (PE Sciex API 5000® or API 5500® Mass Spectrometer; PE Sciex Instruments, Thornhill, Ontario, Canada) with a selected reaction monitoring (SRM) method as follows: moxifloxacin (m/z 402.4→m/z 261.3), levofloxacin (not enantioselective m/z 362.2→m/z 261.1), ciprofloxacin (m/z 332.1→m/z 244.9), linezolid (m/z 338.0→m/z 296.4), clarithromycin (m/z 748.9→m/z 158.4), trimethoprim (m/z 291.2→m/z 230.0), sulfamethoxazole (m/z 254.1→m/z 188.2), doxycycline (m/z 445.2→m/z 428.0), penicillin G (m/z 336.2→m/z 160.0), flucloxacillin (m/z 455.0→m/z 160.2), ampicillin (m/z 350.0→m/z 160.0), amoxicillin (m/z 366.2→m/z 208.1), clavulanic acid (m/z 198.1→m/z 108.1), piperacillin (m/z 518.1→m/z 143.3), tazobactam (m/z 301.1→m/z 122.2), cefazolin (m/z 455.3→m/z 295.3), cefuroxime (m/z 423.0→m/z 207.0), ceftazidime (m/z 547.0→m/z 468.2), meropenem (m/z 384.0→m/z 200.2), caspofungin (m/z 547.4→m/z 538.0), anidulafungin (m/z 1140.8→m/z 1122.2), fluconazole (m/z 307.0→m/z 220.0), voriconazole (m/z 350.4→m/z 127.1), posaconazole (m/z 701.3→m/z 683.6), isavuconazole (m/z 438.2→m/z 224.3). Analyst® software (version 1.6.2, Applied Biosystems/MDS Sciex, Thornhill, Ontario, Canada) was used for the evaluation of chromatograms.

For calibration of the quantitation of antibiotics and antifungals in aqueous samples a calibration curve of five (5) standards was prepared as bulk before starting the project. Using the theoretical concentrations of the standards and measured peak area ratios (peak area of analyte/peak area of internal standard), a linear regression using $1/concentration^2$ as weighting factor was applied, and the correlation coefficient, slope, and intercept were calculated. Calibration standards were prepared by adding the appropriate amount of stock solutions to drug-free

Milli-Q® water (Merck Millipore, Billerica, Massachusetts, USA). For calculation of the dilution factor for calibration standard L1 the density of Milli-Q® water (1.000 g/mL), acetonitrile (0.7820 g/mL), dimethyl sulfoxide (1.1010 g/mL), ethanol (0.7900 g/mL), and methanol (0.7920 g/mL) were used. The response from calibration standards of the different analytes was linear for moxifloxacin: 0.00598–59.8 µg/mL; levofloxacin: 0.00203-20.3 µg/mL; ciprofloxacin: 0.00496-49.6 µg/mL; linezolid: 0.00531-53.1 µg/mL; clarithromycin: 0.0127-127 µg/mL; trimethoprim: 0.00753-75.3 µg/mL; sulfamethoxazole: 0.00902-90.2 µg/mL; doxycycline: 0.00232-23.2 µg/mL; penicillin G: 0.0259-259 µg/mL; flucloxacillin: 0.0151-151 µg/mL; ampicillin: 0.0127-127 µg/mL; amoxicillin: 0.00459-45.9 µg/mL; clavulanic acid: 0.00190-19.0 µg/mL; piperacillin: 0.0186-186 µg/mL; tazobactam: 0.00687-68.7 µg/mL; cefazolin: 0.0282-282 µg/mL; cefuroxime: 0.0111-111 µg/mL; ceftazidime: 0.0302-302 µg/mL; meropenem 0.0127-127 µg/mL; caspofungin 0.0007980-7.98 µg/mL; anidulafungin 0.000769-7.69 µg/mL; fluconazole: 0.00259-25.9 µg/mL; voriconazole: 0.00126-12.6 µg/mL; posaconazole: 0.000695-6.95 µg/mL; and isavuconazole: 0.00177-17.7 µg/mL. The lowest concentration of the calibration ranges was used as quantification limit.

Supplementary Figure 1. Sewage storage in the Patancheru-Kazipally industrial area (when collecting s3)

Supplementary Figure 2. Delivery of industrial effluent water by trucks at the common effluent treatment plant operated by Patancheru Enviro Tech Ltd. (PETL)

Supplementary Figure 3. Musi River in Hyderabad, upstream the Amberpet sewage treatment plant (when collecting s12)

Supplementary Figure 4. Collection of environmental samples at the Rudraram Village Lake (when collecting s8)

Supplementary Figure 5. Pollution on the banks of the Musi River, Hyderabad