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Bacteriophages: A Novel Approach of Treating Multidrug Resistant Bacteria Present In Hospital Wastewater

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Extended Abstract

Abstract

The wide application of antimicrobial agents in clinical settings to treat infectious disease and improper sewage treatment is of great concern to public health as this can lead to the development and evolution of antibiotic resistant bacteria. This occurs as a result of the high selective pressure that antibiotics place on bacteria, resulting in the proliferation and subsequent dissemination of resistant bacteria in the community. We investigated the presence and survival of antibiotic resistant bacteria in untreated hospital wastewaters and their survival after post sewage treatment at three busy hospitals at Davangere, Karnataka. Physiochemical parameters showed high COD levels (552.8 to 714 mg/L), and BOD level observed ranged from 108.6 to 148.4 mg/L. The total heterotrophic bacterial counts, ranged from 2.8 × 105 to 7.3 × 106 CFU/mL, total coliform counts ranged from 0.9 × 103 to 2.4 × 103 MPN/100mL and faecal coliforms count ranged from 110 to 310. In Untreated hospital wastewater high frequency of multidrug resistant bacteria like E. coli, Klebsiella pneumoniae, Enterobacter cloacae, Pseudomonas aeruginosa, Acinetobacter baumannii, Citrobacter freundii, Proteus vulgaris, Staphylococcus aureus and Enterococcus faecium, Salmonella enteritidis and Enterococcus faecalis were isolated. In treated hospital wastewater majority of the bacteria were re-isolated in lesser frequency indicating chlorine is less effective in removal of drug resistant bacteria. Bacteriophages were isolated against all the pathogens from the environment. When untreated hospital wastewater was challenged with the cocktail of bacteriophages, it resulted in 100 % removal of all the multidrug resistant bacteria from the hospital wastewater within 16 hours suggesting bacteriophages could be an alternative to chlorine in wastewater treatment plant. Hence bacteriophage could be an alternative method in treating hospital wastewater when no other substitutes are available, and phage could easily be integrated in sequential batch reactors in wastewater treatment system. Biography VinodKumar C.S has completed his Phd in Microbiology from Gulbarga University, Gulbarga in 2006 and Phd in Medical Microbiology (Virology) in the year 2013 from St. Johns Medical College, Rajiv Gandhi University of Health Sciences, Bangalore. He is the professor of Microbiology, S.S.Institute of Medical Sciences and Research Centre, Karnataka, India. He has over 120 publications that have been cited over 568 times as per google scholar citation, and his publication H-index is 15 and i-10 index is 17 and has been serving as an editor of two journals and board member of reputed Journals.

The wide application of antimicrobial agents in clinical settings to treat infectious disease, as well as their use in aquaculture and veterinary medicine, is of great concern to public health as this can lead to the development and evolution of antibiotic resistant bacteria (Islam, 2011; Mazel and Davis, 1999; Wise at al., 1998). This occurs as a result of the high selective pressure that antibiotics place on bacteria, resulting in the proliferation and subsequent dissemination of resistant bacteria. Resistance genes can also be transferred between cells on plasmids or transposons by transductive or conjugative processes (Berger-Bachi, 2002). DNA elements which mediate integration of resistance genes (eg, integrons) may also be involved (Moura et al., 2011).

Hospitals provide an environment conducive to MDR bacteria, making the treatment options limited and expensive (Magiorakos et al., 2011). Furthermore, not enough is known about their release and survival from hospital wastewater, through the sewerage system and finally into treated effluent released by sewage treatment plants (STPs) into the environment. Sewerage systems also carry other waste materials from the community and industry and so MDR bacteria must survive a long, hostile transition route, including final disinfection, before they are released into surface waters. We proposed that due to their possible persistence in hospitals, MDR bacterial strains could be frequently found in untreated hospital wastewater (UHWW) and, despite the high dilution rate occurring in the sewer systems, these stains may travel to the STP and be detected in both the influent and the treated effluent of the receiving STP. To provide evidence for this hypothesis, we identified bacterial strains found in the untreated wastewater of a hospital in subtropical South East Queensland (SEQ) and traced their movement to the receiving STP to determine if strains survived the sewerage collection and treatment process before treated municipal wastewater is released into the environment.

For this study, we focused on two key pathogens: 1) a Gram-negative bacterium Escherichia coli which, although a common inhabitant of the human gut flora, can also cause several important nosocomial infections such as urinary tract infection, septicaemia and meningitis (Johnson et al., 2005); and 2) a Gram-positive bacterium Staphylococcus aureus which, apart from its well established pathogenicity in hospitalised patients, is also a normal inhabitant of skin of healthy individual and is found in between 25-30% of the interior nares of healthy individuals (Krishna and Miller, 2011). Science Forum and Stakeholder Engagement: Building Linkages, Collaboration and Science Quality.

Methods

Hospital samples were collected from the untreated wastewater outlet pipe of a selected hospital in the subtropical SEQ before it enters the sewer system. The sewer channel taking hospital wastewater to the STP was estimated to be 12.5 km and in view of the high dilution of bacteria in sewage system while travelling to receiving STP, the sampling period was extended for two months to increase the chance of detecting bacterial strains found in UHWW. Using "grab-sampling" technique, water samples were collected for eight consecutive weeks from UHWW and its receiving STP at 10.30am and at 11.00am of the same day respectively. The STP was an activated sludge plant with N and P reduction and services an equivalent population of 130,000 and has a 12-13-day sludge age. Samples were collected from the incoming raw sewage (STP-I) and treated effluent after the activated sludge treatment and chlorination (STP-O). The final effluent is discharged into a nearby waterway. All samples were processed in accordance with the Australian and New Zealand Standards for Water Microbiology and Water Quality Sampling (ANZ standard water microbiology method, 2007). In brief, wastewaters were collected in 500 ml sterile microbiological containers mounted onto a handle of appropriate length. They were transported to the laboratory on ice and processed within 4 hours of collection. Up to 16 E. coli colonies (where possible) were randomly collected from each UHWW sample at each occasion. If samples from the STP outlet were positive for E. coli up to 12 colonies (where possible) were isolated for subsequent fingerprinting. In all, 245 E. coli isolates were isolated from UHWW (n= 120), STP-I (n=102) and STP-O (n= 23). A similar approach was used for S. aureus strains isolated from hospital wastewaters and the STP. In all, 167 S. aureus strains were isolated from UHWW (n=85), STP-I (n=74) and STP-O (n=8). These strains were typed using a high-resolution biochemical fingerprinting method (the PhP-RE system for E. coli and PhP-FS for S. aureus) according to the manufacturer's instruction and RAPD-PCR method as outlined in Naffa et al. (2006). Strains having identical PhP/RAPD pattern were regarded as identical and grouped into common (C) types whilst strains with different PhP and /or RAPD types were regarded as single (S) types. Using the method of Clinical Laboratory Standard Institute (CLSI, 2011), a representative strain of each C-type from UHWW and STP samples was then tested for their antibiotic resistance against nine (for S. aureus) and 16 (for E. coli) antimicrobial agents. For S. aureus, these included tetracycline (30µg), amoxycillin-clavulonic acid (20/10µg), ampicillin (10µg), gentamicin (10µg), ciprofloxacin (5µg), chloramphenicol (30µg), amikacin (30µg), cefoxitin (30µg) and vancomycin (8 µg). For E. coli the antimicrobial agents included tazocin (TZP 55µg), cefotetan (CTT 30µg), cefpodoxime (CPD 10µg), cefoxitin (FOX 30µg), imipenem (IMI 10µg), gentamicin (GEN 10µg), nitrofurantoin (NIT 300µg), trimethoprim (TMP 5µg), sulphafurazole (SF 300µg), sulphamethoxazole (RL 100µg), tetracycline (TET 30µg), ciprofloxacin (CIP 5µg), chloramphenicol (C 30µg), nalixidic acid (NAL 30µg), kanamycin (AK 30µg), and norfloxacin (NOR 10µg).