Antibiotic Resistant Bacteria in Hospital Wastewaters and Sewage Treatment Plants

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Summary

We investigated the presence and survival of antibiotic resistant bacteria in untreated hospital wastewaters (UHWW) and their transmission to the receiving sewage treatment plant (STP) in South East Queensland (SEQ), Australia. Over eight weeks of sampling, 245 Escherichia coli and 167 Staphylococcus aureus strains were collected from UHWW and its receiving STP inlet (STP-I) and post-treatment outlet (STP-O). These strains were typed using the PhP typing method and random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR). Among E. coli strains collected from UHWW, seven common (C) PhP-RAPD types were frequently found in a majority of weekly samples with multi-drug resistant (MDR) C-types also found in STP-I and on three occasions in STP-O. Similarly among S. aureus strains, seven C-types were frequently found in a majority of samples with two MDR C-types also found in STP-I and on two occasions in STP-O. Our data suggest that some MDR bacterial strains found in UHWW may have the ability to survive transmission to the STP and then through to the final treated effluent before being released into surface waters.

Keywords
Multi-drug resistant bacteria, hospital wastewater, sewage treatment plant.

Introduction

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 et 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 (e.g., integrons) may also be involved (Moura et al., 2011) resulting in the further spread of multi-drug resistant (MDR) bacteria.

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 strains 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, septicemia 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).
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 (30 μg), amoxycillin-clavulonic acid (20/10 μg), ampicillin (10 μg), gentamicin (10 μg), ciprofloxacin (5 μg), chloramphenicol (30 μg), amikacin (30 μg), cefoxitin (30 μg) and vancomycin (8 μg). For E. coli the antimicrobial agents included tazocin (TZP 55 μg), cefotetan (CTT 30 μg), cefpodoxime (CPD 10 μg), cefoxitin (FOX 30 μg), imipenem (IMI 10 μg), gentamicin (GEN 10 μg), nitrofurantoin (NT 300 μg), trimethoprim (TMP 5 μg), sulphafurazole (SF 300 μg), sulphamethoxazole (RL 100 μg), tetracycline (TET 30 μg), ciprofloxacin (CIP 5 μg), chloramphenicol (C 30 μg), nalidixic acid (NAL 30 μg), kanamycin (AK 30 μg), and norfloxacin (NOR 10 μg).

Results

Common and Persistent Strains in UHWW and STP

Among E. coli strains collected from UHWW, seven C-Php-RAPD types were frequently found in the majority of the weekly samples. These strains were all MDR and were also found in STP-I and in three occasions in STP-O (Table 1). Strains belonging to these C-types constituted 43% of the isolates (n=52 out of 120) from UHWW. Sixty-eight E. coli isolates from UHWW were found in only one sampling occasion and were regarded as S-types.

Typing of the S. aureus isolates also showed the presence of seven C-Php-RAPD types constituting 65% of the isolates (n=55) in samples collected from UHWW. The remaining 30 isolates were found in only one sampling occasion and were regarded as S-types. Comparison of isolates belonging to C-types with those found in samples collected from the STP (both inlet and outlet) showed the presence of strains with identical Php-RAPD types (Table 1).

Antibiotic Resistance Patterns of Strains from Hospital Wastewater and STP

The pattern of antibiotic resistance among the strains varied in samples collected from different sites. In all, 92% of E. coli strains and 86% of S. aureus strains were resistant to more than two antibiotics (MDR strains), with the highest number being resistant to 12 antibiotics for E. coli (7%) and nine for S. aureus (6%). The mean number of antibiotics to which E. coli strains from the UHWW were resistant to (6.72 ± 2.8) was significantly higher than those found among STP isolates (3.1 ± 1.3) (p<0.0001). Similarly, the mean number of antibiotics to which S. aureus strains from UHWW were resistant to (5.14± 2) was significantly higher than those found in STP isolates (2.9±1.9) (p<0.0001).
Among *E. coli* strains isolated from UHWW, the highest resistance was observed against aztreonam, gentamicin, amoxicillin-clavulanic acid, ceftazidime, cefepime ranging from 89% to 79% with the lowest resistance found against ciprofloxacin; norfloxacin, nalixidic acid, nitrofurantoin and chloramphenicol (0% to 12%).

Among the *S. aureus* strains, the highest resistance was observed against ampicillin followed by amoxicillin/clavulonic acid, gentamicin and cefoxitin ranging from 100% to 78% with the lowest resistance to vancomycin (Figure 1).

![Figure 1. Prevalence of antibiotic resistant *S. aureus* strains isolated from UHWW and its receiving STP inlet and outlet. AMP=ampicillin, AMC=amoxicillin/clavulonic acid, TE=tetracycline, AK=amikacin, CHL=chloramphenicol, CIP=ciprofloxacin, CN=gentamicin, FOX=cefoxitin, VAN=vancomycin.](image)

MDR strains belonging to the same C-types in samples collected from UHWW and STP (both inlet and outlet) typically had identical antibiotic resistance patterns although, on some occasions, strains isolated from STP-O showed less resistance to one (*E. coli* C-type 4) or two (*E. coli* C-type 7) antibiotics.

<table>
<thead>
<tr>
<th>PhP/RAPD C-types</th>
<th>Sites Where Bacteria Were Found</th>
<th>Weeks where Bacteria were Found</th>
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<tbody>
<tr>
<td></td>
<td>UHWW</td>
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<td><em>E. coli</em></td>
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<td>C1</td>
<td>UHWW and STP-I</td>
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<td>C2</td>
<td>UHWW and STP-I and STP-O</td>
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<tr>
<td>C3</td>
<td>UHWW and STP-I</td>
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<td>C4</td>
<td>UHWW and STP-I and STP-O</td>
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<td>C5</td>
<td>UHWW and STP-I</td>
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<td>C6</td>
<td>UHWW and STP-I</td>
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<tr>
<td>C7</td>
<td>UHWW and STP-I and STP-O</td>
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<td><em>S. aureus</em></td>
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<td>C1</td>
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Science Forum and Stakeholder Engagement: Building Linkages, Collaboration and Science Quality Page 227
Significance and Impact

In this study we traced the movement and survival of two important bacterial pathogens, *E. coli* and *S. aureus* from the untreated hospital wastewater to a receiving STP and its discharged effluent. Typing of the isolates using a combination of a high resolution PhP typing and RAPD-PCR confirmed that certain PhP-RAPD types of these bacteria were commonly found in untreated hospital wastewaters. In our study, these strains were regarded as common types. Using the sampling protocol in this study we were able to isolate some of the persistent hospital strains from the inlet of the STP and showed that they belonged to the same PhP-RAPD types.

Originally, we were interested in identifying the presence and survival of antibiotic resistant bacterial strains in both the hospital wastewaters and the STP, but in view of the high level of antibiotic resistance found among both *E. coli* and *S. aureus* strains in hospital wastewaters, and in view of the high diversity of the strains that were only found in one sampling occasion from UHWW (68 *E. coli* S-types and 55 *S. aureus* S-types), we made a comparison between the level of antibiotic resistance found among the strains from hospital wastewaters and the STP. The results indicated that the mean number of antibiotics to which bacteria from hospital wastewater were resistant was significantly higher than those found in the STP receiving these wastewaters. This could partly be due to the fact that hospital wastewater contains as much as 100 times higher antibiotic levels than STPs (Baquero *et al*., 2008; Kummerer 2004).

Interestingly, most of these strains had an identical or very similar antibiotic resistance pattern. Other studies have either identified bacterial strains in hospital wastewater (Ekhaise and Omawoya, 2008; Nunez and Moretton, 2007), or separately in biosolids from an STP (Burtscher and Wuertz, 2003), but have not investigated the movement or survival of these strains to the extent done in our study.

Hospitals present an environment for a concentrated source of resistant bacteria, which may be released into the sewer system. It is therefore important that any study investigating the prevalence of antibiotic resistant bacteria in hospital wastewater consider factors that impact the level of antibiotic resistant bacteria in such wastewaters. For instance, it is possible that some of these antibiotic resistant bacteria are sourced from community effluents upstream of the hospital since the bulk of antibiotic treatment in the community would occur at home. In this study, we did not test any samples from hospital upstream effluents to rule out this possibility but Galvin *et al.* (2010) have shown that hospital wastewater had a higher proportion of antibiotic resistant *E. coli* than wastewater upstream of the hospital; and that the sewage treatment process was effective at removing the sensitive *E. coli* strains but failed to remove a number of antibiotic resistant strains (Galvin *et al*., 2010). The original aim of sewage treatment was not for pathogen control, yet we now know that STPs do significantly reduce waterborne pathogen loads in the community.

In our study, we found a high reduction in the antibiotic resistant strains in hospital wastewater by the time they reached the STP. Interestingly, the few surviving strains that appeared to survive hospital discharge and sewage treatment were those that not only were MDR but also belonged to common types and were frequently found in the hospital wastewater at different weeks. We postulate that these common types may constitute the hospital persistent strains that have evolved to persist in the environment, including final chlorination after sewage treatment.

We did not compare the prevalence of antibiotic resistant bacteria found in our samples with those found in a treated hospital wastewater. Several studies have investigated the treatment of hospital wastewater by quantitatively comparing two different treatment methods (i.e., conventional activated sludge system and plate membrane bioreactor) of hospital effluent treatment (Pauwels *et al*., 2006). However, the results indicate that these treatment systems are not always effective against all types of bacteria present in the wastewater from hospitals. For instance, a Brazilian study investigated a hospital sewage treatment plant that used an extended aeration activated sludge process and showed that the treatment of the hospital wastewater may not be totally effective in removing antibiotic resistant bacteria and resistance genes from the hospital wastewater (Chagas *et al*., 2011). This could partly be due to the ability of MDR bacterial to survive not only the hospital environment but also the municipal wastewater once they are released from hospitals. Such MDR strains, if they gain residency in hospitals, can be constantly released in hospital wastewater as shown in our study. Based on these findings, it can be postulated that on-site treatment of hospital wastewater would have little impact on the presence of MDR bacteria in municipal wastewater. This however, has yet to be fully investigated before any conclusion can be made.

A general conclusion and observation presented here is that we found the presence of MDR *E. coli* and *S. aureus* strains belonging to a few common types in untreated hospital wastewater. These strains were able to survive the journey to the inlet of the STP. However, only strains of certain types were able to survive treatment process of the STP, including chlorination, to be released into the surface waters. These strains in our study were shown to be frequently present in hospital wastewater suggesting their persistence in the hospital environment. The significance of this for public health however, requires further work to fully characterise and quantify the input of MDR bacteria from hospitals compared with those originating in the general community or other wastewater-related sources.
References

Australian and New Zealand Standards. 2007. Australian/New Zealand Standard Water Microbiology method 1: General information and procedures. AS/NZS 4276.1


