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RESEARCH ARTICLE

Frequency of transferable multiple antibiotic resistance amongst coliform bacteria isolated from a treated sewage effluent in Antofagasta, Chile

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Abbreviations: EMB: eosin methylene blue agar

Antibiotic-resistant coliform bacteria from raw and treated water from activated sludge of a sewage treatment plant were isolated on eosin methylene blue agar (EMB) plates in the separate presence of ampicillin, tetracycline, chloramphenicol and streptomycin. Antibiotic resistant coliforms obtained from inflow and effluent waters were studied to determine influences of treatment on the species composition, resistance patterns and transferable resistance in these bacteria. The bacterial counts obtained showed a high proportion of antibiotic resistant coliforms in the treated effluent. The resistant coliform species were eliminated at different rates by the treatment. *Escherichia coli* decreased, whereas the proportion of *Klebsiella* sp increased. The percentage of multiple-resistant isolates increased at the end of the treatment process. The high frequency of resistance was to ampicillin, followed by tetracycline and sulpha-trimethoprim. Moderate incidence of resistance was to streptomycin, chloramphenicol and cephradine. The

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lowest rate of resistance was to kanamicin and nalidixic acid. Mating experiments of multiple-resistant coliforms with an *E. coli* K-12 donor confirmed the capability of transferring its resistance. The results of this study suggested that wastewater treatment could reduce the total number of enteric bacteria in sewage, but may increase the proportion of antibiotic resistant coliforms in effluent water. Environmental release and agricultural use of treated sewage effluent containing bacteria having elevated antibiotic resistance levels presents a potential public health risk, as well as the risk of transferring the antibiotic resistance to bacterial populations in the environment.

During recent decades, antibiotics have been widely used as therapy for bacterial infections in humans and animals, and as growth promoters in agriculture and aquaculture (Kruse and Sorum, 1994; Khachatourians, 1998; Aarestrup, 1999), increasing the percentages of antibiotic resistant bacteria in various environments (Pathak et al. 1993; Young, 1993; Guardabassi et al. 1998; van den Bogaard and Stobberingh, 1999) and may cause problems in therapy by selecting resistant bacteria (Levy, 1998).

The occurrence of antibiotic resistant bacteria in the aquatic environment has been demonstrated in many studies (Andersen and Sandaa, 1994; Miranda and Castillo, 1998; Boon and Cattanach, 1999; Huys et al. 2001) as a consequence of uncontrolled discharges urban and animal wastewater (Guardabassi et al. 1998; Goñi et al. 2000). For this reason, many investigators have recognized that wastewater treatment plants are the principal recipients of enteric bacteria with multiple antibiotic resistance (Iversen et al. 2002; Selvaratnam and Kunberger, 2004) and an important site for horizontal gene transfer, by containing nutrients and high concentrations of microorganisms (Goni-Urriza et al. 2000; Barberio et al. 2001; Vilanova et al. 2004). Release of genetically engineered microorganisms to public wastewater treatment plants may result in dissemination of deleterious genes into the environment with potentially adverse effects to public health (McClure et al. 1991). Andersen (1993) has called for a re-evaluation of water quality standards based on the above considerations.

Biological treatment processes at sewage treatment plants could produce selective elimination, and/or changes in the proportions of phenotypes within effluent bacterial populations (Andersen, 1993; Mezrioui and Baleux, 1994). Furthermore the disposal of treated sewage into rivers, lakes, or elsewhere may or may not influence environmental bacterial populations (Vilanova et al. 2002). Some studies have found that wastewater treatment can raise or lower the proportions of antibiotic resistant bacteria which carry antibiotic resistance plasmids (Andersen, 1994; Ohlsen et al. 2003).

The objective of the present study was to examine the influence of sewage treatment in a local plant on the presence of antibiotic resistance in coliform bacteria in the water processed in order to determine: i) the influence of the treatment plant on bacterial counts in the effluent, ii) the species composition of the antibiotic resistant coliforms in the effluent, iii) the incidence of antibiotic resistance among bacteria in the effluent, and iv) the transferable resistances within antibiotic resistant bacteria in the effluent.

MATERIALS AND METHODS

Sample Collection

Samples of wastewater were collected from an activated sludge system at an urban sewage treatment plant, located in Antofagasta, Chile. This plant has a biological carrying capacity of 100,000-120,000 person-equivalents. The sewage spends a total retention time in the system of about 3-4 hrs prior to the (final) chlorination step, with a discharge rate of 50 L/sec of treated effluent. Duplicate samples of raw and outfall water were collected in autoclaved 1-L bottles, held at 4ºC during transportation to the laboratory and assayed within 2 hrs of collection. Samples were collected on four occasions, including September and December 2001, and March and July 2002.

Total coliform and antibiotic resistant coliform counts

Serial dilutions of both raw and treated water samples were plated by spreading 0.1 ml on eosin methylene blue agar

Table 1. Bacterial counts and percentages of antibiotic resistant coliforms on EMB agar in water samples collected from raw and treated sewage on four different sampling dates, at the Antofagasta sewage treatment plant.

Date	Samples	Ampicillin		Tetracycline		Chloramphenicol		Streptomycin		Total Coliforms
		CFU/ml	%	CFU/ml	%	CFU/ml	%	CFU/ml	%	
September 2001	Raw	2500	2,8	190	0.2	850	1.0	750	0.9	87000
	Treated	20	13,3	5	3.3	3	2.0	11	7.3	150
December 2001	Raw	2900	1.7	1400	0.8	1800	1.1	1900	1.1	170000
	Treated	14	17.9	7	9.0	5	6.4	10	12.8	78
March 2001	Raw	4900	1.8	7500	0.3	670	0.2	640	0.2	270000
	Treated	30	11.5	5	1.9	3	1.2	9	3.5	260
July 2002	Raw	2400	1.3	180	0.1	530	0.3	420	0.2	180000
	Treated	20	7.4	3	1.3	4	1.7	7	3.0	230

The differences of the proportions of resistant bacterial counts between raw and treated sewage is significant $P < 0,05$.

(EMB; Difco) for total coliform counts. Plates were incubated at 37ºC for 18-24 hrs and bacterial counts were expressed as CFU/ml.

Antibiotics were individually added into EMB plates to obtain counts of antibiotic resistant coliforms. The plates were prepared with fresh antibiotic solutions to give final concentrations as follows: ampicillin, 50 µg/ml; tetracycline, 25 µg/ml; chloramphenicol (25 µg/ml) and streptomycin, 25 µg/ml. Then 0.1 ml of appropriate dilutions of samples were spread on the above selective media and the lactose positive colonies were counted after 24-48 hrs of incubation at 37ºC.

Identification of antibiotic resistant coliforms

A maximum of 34 lactose positive colonies were selected from raw and treated sewage samples plated on EMB-antibiotic agar, and biochemical characterization of the isolates was performed to identify species by a standard procedure used for *Enterobacteriaceae* (Kelly et al. 1995). The identification included the following biochemical tests: indol, urea, citrate, TSI, LIA and MIO (Difco). Isolates were maintained in glycerol broth at -20ºC for further study.

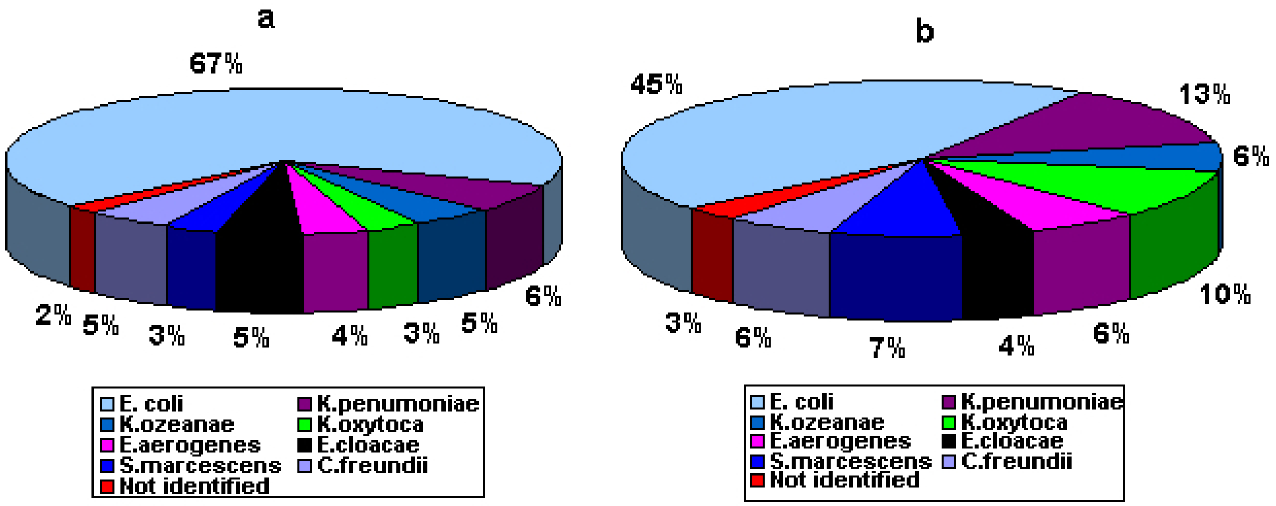
Determination of antibiotic resistance

A total of 248 coliform isolates from raw and treated sewage were tested for antibiotic resistance. It was determined by a standard disc diffusion technique using Mueller-Hinton agar (Difco) according with the recommendations of National Committee for Clinical Laboratory Standards (NCCLS, 2000). The antimicrobial drugs tested and their sensidisk concentrations were: ampicillin (AM), 10 µg; cephradine (CE), 30 µg; chloramphenicol (CM), 30 µg; kanamicin (KM), 30 µg; nalidixic acid (NA), 30 µg; streptomycin (SM), 10 µg; sulphametoxazol-trimethoprim (SXT), 1.25/23.75 µg; and tetracycline (TE) (30 µg). The tests were performed following NCCLS (2000) recommendations, including Escherichia coli ATCC 25922 as a control strain. Patterns of antibiotic resistance were determined, reviewing the order of importance (as %) of each type of resistance for each sample evaluated (Andersen and Sandaa, 1994).

Transference of bacterial resistance. Conjugation experiments were carry out in broth, as previously described by Silva et al. (1987). Some multi-resistant coliform strains NA^S isolated from treated sewage were used as donor organisms, and *Escherichia coli* K-12 Lac$^-$ NA^R, as a recipient strain. Both donor and recipient were cultivated in Mueller-Hinton broth a 37ºC over night and diluted approximately 6 x 10^8 cells per ml. One millilitre of the donor broth was mixed with 1 ml of the recipient broth in 4 ml of MH broth and incubated at 37ºC for 24 hrs. After 0.1 ml of the conjugation mixtures were spread on McConkey agar containing 25 µg of NA and one of the following antibiotics: ampicillin, cephradine, chloramphenicol, kanamycin tetracycline and sulfaphametoxazo-trimethoprim (25 µg) to select transconjugants. Then transconjugants were subcultured on non-selectives medium and the resistance pattern was determined by the disc dilution technique.

Statistical analysis. The antibiotic resistances are expressed as percentage. The statistical significance of differences in antibiotic resistance for row and treated sewage was evaluated using the Comparisons of Proportions Analysis. An ANOM (analysis of means) statistical technique for proportions data was used to determine statistical significance. The null hypothesis that

Figure 1. Distribution of the species of resistant coliforms found in sewage samples.
(a) Raw sewage.
(b) Treated sewage.



the proportions of antibiotic resistant coliforms were equal for row and treated sewage. A *P* value equal to or less than 0,05 was considered significant.

RESULTS

Table 1 presents results obtained on the bacterial plate counts from raw and treated water at the Antofagasta plant, showing decreases in coliform counts after treatment of few orders of magnitude. Table 2 shows that in results from the treated water the resistant coliform counts were higher on the plates with ampicillin than with other antibiotics and that the percentage of resistant coliforms increased significantly in treated water, particularly in the austral summer month of December ($P < 0,05$). The lowest percentages of resistant isolates from treated water were observed in the austral winter month of July. Coliform bacteria exhibited the highest incidence of resistance to ampicillin, followed by streptomycin and tetracycline, and the lowest resistance was for chloramphenicol (Table 2).

Eight different species were identified by the biochemical tests, with *E. coli* the most frequent species isolated from both raw and treated sewage (Figure 1). In raw water, *E. coli* formed 67% of the total isolates, decreasing to 45% of the isolates in treated water. Three *Klebsiella* species (*K. pneumoniae*, *K. oxytoca* and *K. ozaenae*) presented a frequency of 14% in raw water, increasing to 29% in treated water isolates. The frequencies of other species identified presented smaller changes. A small proportion of isolates remained unidentified.

The percentages of resistance to different antibiotics in coliform species increased significantly in isolates from treated water against all antibiotics (Table 2). These results showed a great variability of resistance in the coliform species. The most resistant species were *Klebsiella* spp isolates from raw water and *Serratia* spp isolates from treated water. Figure 2 summarizes the total frequency of antibiotic resistance for all coliform isolates. A high resistance was found to ampicillin, with 85% in isolates from raw water and 95% in treated water, followed by tetracycline and sulphamethoxazole-trimethoprim. Moderate resistance was observed to streptomycin, chloramphenicol and cephradine. The lowest resistance was observed with kanamycin and nalidixic acid.

Resistant coliforms from raw and treated sewage show a wide range of multiresistance distribution, exhibiting resistance to as many as 8 antibiotics. The frequency of multiple resistance was always higher in treated sewage than in raw sewage, as presented in Table 3.

Transferable determinants of antibiotic resistance were found in coliform species from treated sewage by total or partial transference of its antibiotic-resistance patterns into *E. coli* K-12 (Table 4).

DISCUSSION

The results of this study showed the occurrence of antibiotic resistant coliforms in both raw and treated wastewater from an activated sludge treatment plant. A significant increase was found in the percentage of resistant coliforms in treated water in agreement with reports by other investigators (Reinthaler et al. 2003). The counts of resistant coliforms encountered in the Antofagasta treatment plant were higher than that described by other investigators (Andersen and Sandaa, 1994). The high values of resistance found to ampicillin was in general agreement with that reported by others (Andersen and Sandaa, 1994; Boon and Cattanach, 1999), and is very common in coliforms isolated from the human and animal intestines. The occurrence of coliforms with high resistance to ampicillin and other antibiotics reflect human influence in the environment (Andersen and Sandaa, 1994). Climatic conditions in summer months probably influenced increases the proportion of resistant coliforms as demonstrated in our study, as shown by the higher percentages of resistant isolates observed in the December samples. Also, several investigators have suggested that chlorination selects or induces changes in antibiotic resistance in bacterial populations. The resistant coliforms may have better survival advantages than antibiotic-sensitive coliforms in treated wastewater.

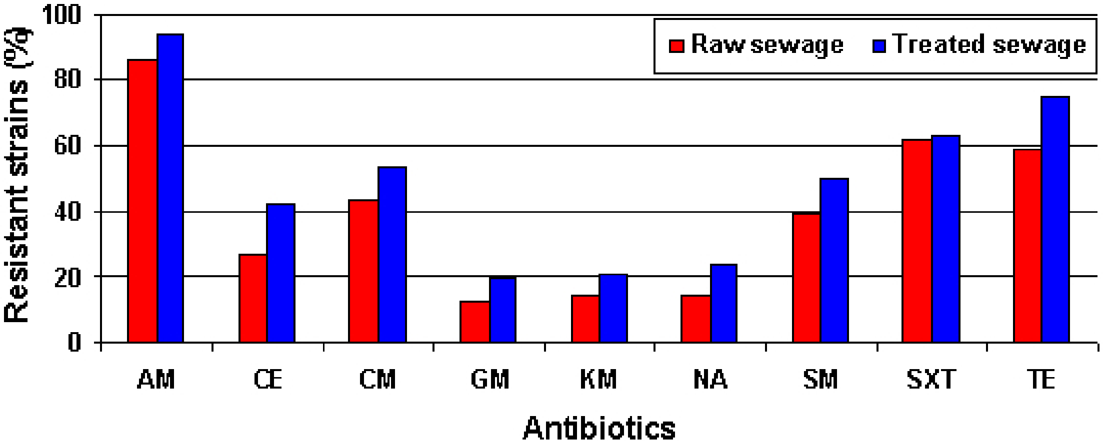
In a recent study, it was found that discharges of treated sewage effluent did not effect the load, structure and

Table 2. Percentages of resistance to different antibiotics in coliform species isolated from raw and treated sewage from the Antofagasta sewage treatment plant.

Antimicrobial Drugs	*Escherichia coli*		*Enterobacter* spp		*Klebsiella* spp		*Citrobacter* spp		*Serratia* spp	
	Raw n=100	Treated n=46	Raw n=13	Treated n=10	Raw n=20	Treated n=29	Raw n=7	Treated n=6	Raw n=4	Treated n=7
Ampicillin	74	89*	85	90	90	96	86	100	75	100
Nalidixic acid	15	24	8	10	25	21	14	17	25	43
Cephradine	22	35*	54	80*	15	41	28	50	25	29
Chloramphenicol	43	50	15	30	30	45	71	67	50	71
Streptomycin	44	54	23	40	55	58	29	50	50	71
Gentamicin	4	15*	8	30	5	17	14	17	25	43
Kanamicin	17	26	15	20	15	31	14	17	25	43
Cotrimoxazole	65	78	15	20	70	79	71	67	75	71
Tetracycline	67	72	38	50	75	86	57	83	50	71

*The difference in proportions of the resistant coliform species between raw and treated sewage is significant for some species ($P < 0,05$).

Figure 2. Comparison of antimicrobial resistance on 248 coliforms strains isolated from raw and treated sewage of the treatment plant, Antofagasta. The increase of antibiotic resistance in coliforms of treated sewage is significative ($P < 0,05$) to all antibiotics, except to sulfametoxazol-trimetoprim (SXT).



composition of faecal coliforms in the receiving river (Vilanova et al. 2002). However, when the identification of resistant coliform species in raw and treated waters are made, significant differences are found in species composition. *E. coli* was removed more efficiently from the sewage than other species, with about thirty percent of its initial population eliminated at the end of the treatment. Andersen (1993) reported that total *E. coli* was reduced by about half its initial number on its way through the treatment plant. In both cases, the reduction of *E. coli* was significant; existing differences may be explained due to the presence in our study of antibiotic-resistant *E. coli* which may be able to survive better than antibiotic-sensitive strains. On the other hand, *Klebsiella* sp isolates were not removed more efficiently than *E. coli* and their percentage increased by 2X in treated wastewater. It is evident that *Klebsiella* sp. were more resistant to the treatment procedure than *E. coli*. In the hospital environment, *Klebsiella* sp is a causative agent of several kinds of infections and many of the nosocomial infections are caused by multiresistant species (Gonzalez-Vertiz et al. 2001). Also, it exhibits characteristics in cellular structure, including presence of a capsular polysaccharide, which may permit these species to achieve better survival through the treatment procedure.

Several investigations on antibiotic resistant enteric bacteria in wastewater treatment plants have led to contradictory conclusions. Some investigators reported that antibiotic resistant bacteria were removed less efficiently than antibiotic sensitive organisms (Iwane et al. 2001). Others concluded that elimination of total coliforms and resistant coliforms were similar (Walter and Vennes, 1985). Guardabassi et al. (2002) reported that coliforms and *Acinetobacter* spp. from treated water and digested sludge were generally not significantly more resistant compared with isolates from raw water exposed to tertiary wastewater treatment. Finally, it was found that resistance among sewage bacteria during the treatment procedure can be either decreased or increased (Andersen, 1993), while the present study found that although the total number of coliforms was reduced considerably, the proportion of antibiotic resistant coliforms in treated water increased. It is possible that antibiotic resistant coliforms have ecological advantages over antibiotic sensitive coliforms in an activated sludge sewage plant.

Table 3. Percentages antibiotic resistance in 248 coliform isolates to one or more antibiotics from raw and treated sewage.

N° of Resistant Determinants (Antibiotics)	N° of Isolates		% of Antibiotic Resistant Isolates	
	Raw	Treated	Raw	Treated
1	40	15	27	15
2	25	13	17	13
3	19	16	13	16
4	21	18	14	16
5	21	15	14	15
6	6	13	4	13
7	4	4	3	4
8	11	6	8	6

Explanations for the differences in removal of bacteria is that sewage treatment plants have varying degrees of efficiency in elimination of bacteria due to the type of treatment plant, the initial sewage composition, and the treatment procedure, which can affect changes in the final sewage composition. The Antofagasta treatment plant is apparently working well, reducing total coliform and resistant coliforms to acceptable levels from a sanitary standpoint, but the release of treated water containing bacteria having increased antibiotic resistance levels is an undesirable outcome, which should be remediated (Andersen, 1993).

The observation of increased resistance frequency to ampicillin, tetracycline, streptomycin and chloramphenicol after wastewater treatment has previously been reported by Andersen (1993), and Reinthaler et al. (2003). In the present study, the resistance to ampicillin was notably high, reaching around 90% in coliforms from treated water and it is possibly related to the presence of beta-lactamase in the cells (Silva et al. 1987). A smaller proportion of resistance was observed to tetracycline 80%, sulpha-trimethoprim 78% and over 40% to streptomycin, chloramphenicol and cephradine. Also, multiple antibiotic resistance was detected in isolates from both raw and treated water, but the frequency of resistance to 3, 4, 5, 6, 7, and 8 antibiotics was increased in coliforms from treated water. Similar results have been reported in coliform isolates from sewage treatment plant (Andersen, 1993).

The bacterial conjugation experiments showed that a high percentage of multi-resistant coliforms tested (89%) were able to partially or completely transfer their resistance patterns to *E. coli* K-12. This suggested that a small percentage of the resistance had chromosomal origin and a high proportion of the multi-resistance was mediated by conjugative plasmids, which were easily transferred to *E. coli* K-12. These findings agree with other studies which established that sewage treatment plants are potential sites for the genetic transfer of antibiotic resistance in some species of bacteria (Andersen, 1993; Goñi-Urriza et al. 2000; Schluter et al. 2003). The increase of multi-resistance in coliforms isolated from treated wastewater is explainable by bacterial conjugation or other genetic mechanisms of change from antibiotic resistant to antibiotic sensitive bacteria by horizontal gene transfer within the treatment plant.

It is possible that factors such as pH, temperature, salts, nutrient composition, concentration of donor and recipient bacteria of transferable genes, may affect the ability of plasmid transfer, but gene transfer may still be possible in different steps of the treatment procedure (Andersen, 1993).

The wastewater treatment plant constitutes an important reservoir of enteric bacteria which carry potentially transferable resistance genes. This study has demonstrated that sewage treatment may result in the increase the proportion of antibiotic resistant bacteria in effluent water. The use of the treated sewage may contribute to spread antibiotic resistance in the environment, specially when regions poor in water resources re-use it in watering agricultural crops, such as in northern part of Chile. This represents a dangerous public health risk, which needs future evaluation and control.

REFERENCES

AARESTRUP, Frank Møller. Association between the consumption of antimicrobial agents in animals husbandry and the occurrence of resistant bacteria among food animals. *International Journal of Antimicrobial Agents*, August 1999, vol. 12, no. 4, p. 279-285.

ANDERSEN, Sigrid Rita. Effects of waste water treatment on the species composition and antibiotic resistance of coliform bacteria. *Current Microbiology*, February 1993, vol. 26, no. 2, p. 97-103.

ANDERSEN, Sigrid Rita and SANDAA, R.A. Distribution of tetracycline resistance among gram-negative bacteria isolated from polluted and unpolluted marine sediments. *Applied and Environmental Microbiology*, March 1994, vol. 60, no. 3, p. 908-912.

BARBERIO, Claudia; PAGLIAI, Letizia; CAVALIERI,

Table 4. Antibiotic resistance patterns of coliform strains recovered from treated sewage and their transference to *Escherichia coli* K-12 (transconjugants).

Donor Organisms	N° of Strains	Resistance Pattern	N° of Transconjugants	Transferred Pattern
E. coli	1	AM-TE-CM	1	AM
E. coli	2	AM-TE-SXT	2	AM-TE
E. coli	1	AM-TE-SM-KM	1	AM-TE
			1	AM-TE-KM
E. coli	2	AM-TE-SM-SXT	1	AM-TE-SM-SXT
E. coli	4	AM-TE-CM-SM-SXT	3	AM-TE-CM
			1	AM-TE-CM-SM
K. oxytoca	1	AM-TE-SXT	1	AM-TE
K. oxytoca	1	AM-CM-SXT	1	AM-CM
K. oxytoca	2	AM-TE- SXT-SM	1	AM-TE-SXT
			1	AM-TE-SXT-SM
K. oxytoca	1	AM-TE- SXT-SM-CM-KM	1	AM-TE-SM
			1	AM-TE-SM-SXT-CM
K. ozaenae	2	AM-TE- SXT-SM	1	AM-TE
			1	AM-TE-SM-SXT
E. aerogene	1	AM-TE-SXT	1	AM-TE-SXT
E. aerogene	1	AM-TE-CE	1	AM-TE
C. freundii	1	AM-CM-SXT	1	AM-CM-SXT
C. freundii	1	AM-CM-SXT-TE-SM	1	AM-CM-SXT-TE-SM

Duccio and FANI, Renato. Biodiversity and horizontal gene transfer in culturable bacteria isolated from activated sludge enriched in nonylphenol ethoxylates. *Research in Microbiology*, January-February 2001, vol. 152, no. 1, p. 105-112.

BOON, P.I. and CATTANACH, M. Antibiotic resistance of native and faecal bacteria isolated from rivers, reservoirs and sewage treatment facilities in Victoria, south-eastern Australia. *Letters in Applied Microbiology*, March 1999, vol. 28, no. 3, p. 164-168.

GONZALEZ-VERTIZ, Artemio; ALCANTAR-CURIEL, Dolores; CUAUHTLI, Marcela; DAZA, Carlos; GAYOSSO, Catalina; SOLACHE, Graciela; HORTA, Cruz; MEJIA, Francisco; SANTOS, Jose I. and ALPUCHE-ARANDA, Celia. Multi-resistant extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* causing an outbreak of nosocomial bloodstream infection. *Infections Control Hospital Epidemiology*, November 2001, vol. 22, no. 11, p. 723-725.

GOÑI-URRIZA, Marisol; CAPDEPUY, Michèle; ARPIN, Corinne; RAYMOND, Nathalie; CAUMETTE, Pierre and QUENTIN, Claudine. Impact of an urban effluent on antibiotic resistance of riverine *Enterobacteriaceae* and *Aeromonas* spp. *Applied and Environmental Microbiology*, January 2000, vol. 66, no. 1, p. 125-132.

GUARDABASSI, Luca; PETERSEN, Andreas; OLSEN, John E. and DALSGAARD, Anders. Antibiotic resistance in *Acinetobacter* spp. Isolated from sewers receiving waste effluent from a hospital and a pharmaceutical plant. *Applied and Environmental Microbiology*, September 1998, vol 64, no. 9, p. 3499-3502.

GUARDABASSI, Luca; LO FO WONG, Danilo M.A. and DALSGAARD, Anders. The effects of tertiary wastewater treatment on the prevalence of antimicrobial resistant bacteria. *Water Research*, April 2002, vol. 36, no. 8, p. 1955-1964.

HUYS, G.; GEVERS, D.; TEMMERMAN, R.; CNOCKAERT, M.; DENYS, R.; RHODES, G.; PICKUP, R.; MCGANN, P.; HINEY, M.; SMITH, P. and SWINGS, J. Comparison of the antimicrobial tolerance of oxytetracycline-resistant heterotrophic bacteria isolated from hospital sewage and freshwater fishfarm water in Belgium. *Systematic and Applied Microbiology*, April 2001, vol. 24, no. 1, p. 122-130.

IVERSEN, Aina; KÜHN, Inger; FRANKLIN, Anders and MÖLLBY, Roland. High prevalence of vancomycin-resistant enterococci in Swedish sewage. *Applied and Environmental Microbiology*, June 2002, vol. 68, no. 6, p. 2838-2842.

IWANE, T.; URASE, T. and YAMAMOTO, K. Possible impact of treated wastewater discharge on incidence of antibiotic resistant bacteria in river water. *Water Science and Technology*, January 2001, vol. 43, no. 2, p. 91-99.

KELLY, M.T.; BRENNER, J. and FARMER III, J.J. Enterobacteriaceae. In: HAUSLER, William J.; HERRMANN, Kenneth L.; ISENBERG, Henry D. and SHADOMY, H. eds. *Manual of Clinical Microbiology*. 4th ed. American Society for Microbiology, Washington DC, 1995, p. 347-352.

KHACHATOURIANS, G.G. Agricultural use of antibiotics and the evolution and transfer of antibiotic-resistant bacteria. *Canadian Medical Association Journal*, November 1998, vol. 159, no. 9, p. 1129-1136.

KRUSE, H. and SORUM, H. Transfer of multiple drug resistance plasmids between bacteria of diverse origins in natural microenvironments. *Applied and Environmental Microbiology*, November 1994, vol. 60, no. 11, p. 4015-4021.

LEVY, Stuart B. The challenge of antibiotic resistance. *Scientific American*, March 1998, vol. 278, no. 3, p. 46-53.

MEZRIOUI, N. and BALEUX, B. Resistance patterns of *E. coli* strains isolated from domestic sewages before treatment in both aerobic lagoon and activated sludge. *Water Research*, November 1994, vol. 28, no. 11, p. 2399-2406.

MCCLURE, N.C.; FRY, J.C. and WEIGHTMAN, A.J. Survival and catabolic activity of natural and genetically engineered bacteria in a laboratory-scale activated-sludge unit. *Applied and Environmental Microbiology*, February 1991, vol. 57, no. 2, p. 366-373.

MIRANDA, C.D. and CASTILLO, G. Resistance to antibiotic and heavy metals of motile aeromonads from Chilean freshwater. *The Science of the Total Environment*, December 1998, vol. 224, no. 1-3, p. 167-176.

NATIONAL COMMITTEE FOR CLINICAL LABORATORY STANDARDS (NCCLS). *Performance standards for antimicrobial disk susceptibility test-approved standard (document M2-A7)*. 7th ed. NCCLS, Pennsylvania, USA, 2000. ISBN 1-56-238208-X.

OHLSEN, Knut; TERNES, Thomas; WERNER, Guido; WALLNER, Ursulla; LÖFFLER, Dirk; ZIEBUHR, Wilma; WITTE, Wolfgang and HACKER, Jörg. Impact of antibiotics on conjugational resistance gene transfer in *Staphylococcus aureus* in sewage. *Environmental Microbiology*, August 2003, vol. 5, no. 8, p. 711-716.

PATHAK, S.P.; BHATTACHERJEE, J.W. and RAY, P.K. Seasonal variation in survival and antibiotic resistance among various bacterial populations in a tropical river. *Journal of General and Applied Microbiology*, March 1993, vol. 39, no. 3, p. 47-56.

REINTHALER, F.F.; POSCH, J.; FEIERL, G.; WÜST, G.; HAAS, D.; RUCKENBAUER, G.; MASCHER, F. and MARTH, E. Antibiotic resistance of *E. coli* in sewage and sludge. *Water Research*, April 2003, vol. 37, no. 8, p. 1685-1690.

SCHLÜTER, A.; HEUER, H.; SZCZEPANOWSKI, R.; FORNEY, L.J.; THOMAS, C.M.; PÜHLER, A. and TOP, E.M. The 64 508 bp IncP-1beta antibiotic multiresistance plasmid pB10 isolated from a waste-water treatment plant provides evidence for recombination between members of different branches of the IncP-1beta group. *Microbiology*, November 2003, vol. 149, no. 11, p. 3139-3153.

SELVARATNAM, Shivi and KUNBERGER, J.D. Increased frequency of drug-resistant bacteria and fecal coliforms in an Indiana Creek adjacent to farmland amended with treated sludge. *Canadian Journal of Microbiology*, August 2004, vol. 50, no. 8, p. 653-656.

SILVA, J.; ZEMELMAN, R.; MANDOCA, M.A.; HENRÍQUEZ, M.; MERINO, C. and GONZÁLEZ, C. Antibiotic- resistant gram negative bacilli isolated from sea water and shellfish. Possible epidemiological implications. *Revista Latinoamericana de Microbiología*, April-June 1987, vol. 29, no. 2, p.165-169.

VAN DEN BOGAARD, A.E. and STOBBERINGH, E.E. Antibiotic usage in animals: Impact on bacterial resistance and public health. *Drugs*, October 1999, vol. 58, no. 4, p. 589-607.

VILANOVA, X.; MANERO, A.; CERDÀ-CUÉLLAR, M. and BLANCH, A.R. The effect of a sewage treatment plant effluent on the faecal coliforms and enterococci populations of the reception river waters. *Journal of Applied Microbiology*, February 2002, vol. 92, no. 2, p. 210-214.

VILANOVA, X.; MANERO, A.; CERDÀ-CUÉLLAR, M. and BLANCH, A.R. The composition and persistence of faecal coliforms and enterococcal populations in sewage treatment plants. *Journal of Applied Microbiology*, February 2004, vol. 96, no. 2, p. 279-288.

WALTER, M.V. and VENNES, J.W. Occurrence of multiple-antibiotic-resistant enteric bacteria in domestic sewage and oxidation lagoons. *Applied and Environmental Microbiology*, October 1985, vol. 50, no. 4, p. 930-933.

YOUNG, Hilary-Kay. Antimicrobial resistance spread in aquatic environments. *The Journal of Antimicrobial Chemotherapy*, May 1993, vol. 31, no. 5, p. 627-635.
