ORIGINAL ARTICLE

Detection of extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* in effluents and sludge of a hospital sewage treatment plant

T. Prado[1], W.C. Pereira[1], D.M. Silva[1], L.M. Seki[2], A.P.D'A. Carvalho[2] and M.D. Asensi[2]

1 Department of Sanitation and Environmental Health – Public Health National School, Oswaldo Cruz Foundation – Rio de Janeiro (RJ), Brazil
2 Department of Bacteriology – Oswaldo Cruz Institute, Oswaldo Cruz Foundation — Rio de Janeiro (RJ), Brazil

Keywords
antimicrobial resistance, ESBL, hospital sewage effluent, *K. pneumoniae*.

Correspondence
Marise Dutra Asensi, Department of Bacteriology/IOC/FIOCRUZ, Av. Brasil, 4365, 21045-900, Rio de Janeiro, RJ, Brasil.
E-mail: marise@ioc.fiocruz.br

2007/1252: received 6 August 2007, revised 19 September 2007 and accepted 20 September 2007

doi:10.1111/j.1472-765X.2007.02275.x

Abstract

Aims: To detect ESBL (extended-spectrum β-lactamase)-producing *Klebsiella pneumoniae* present in the effluents and sludge of a hospital sewage treatment plant, evaluating the treatment plant's potential to remove these micro-organisms.

Methods and Results: Twenty samples (crude sewage, UASB reactor effluent, filtered effluent and sludge) were collected in the period from May to December 2006, in order to analyse antimicrobial susceptibility and to check ESBL production, the disc-diffusion and the combined disc methods were used. Total and faecal coliform concentrations were also determined. ESBL-producing *K. pneumoniae* were detected in all samples analysed, representing 46·5% of the total strains isolated. Among the non-ESBL-producing strains, 26% were multiresistant and one strain resistant to eight of the nine antimicrobials tested was detected in the treated effluent.

Conclusions: The hospital wastewater treatment plant did not show a satisfactory efficacy in removing pathogenic micro-organisms, allowing for the dissemination of multiresistant bacteria into the environment.

Significance and Impact of the Study: The inefficacy of hospital wastewater treatment plants can result in routes of dissemination of multiresistant bacteria and their genes of resistance into the environment, thus contaminating water resources, and having serious negative impact on public health.

Introduction

Wastewaters coming from health care institutions may contain several toxic substances and pathogenic micro-organisms, including radioisotopes, hormones, drugs, heavy metals and antimicrobial-resistant bacteria (Kummerer 2001; Reinthaler *et al.* 2003). Failure to treat these effluents and the lack of basic sanitation services may contribute to the establishment of routes of dissemination of these micro-organisms and of their resistance genes into the environment (Meirelles-Pereira *et al.* 2002).

Many resistant bacteria and resistance genes have been detected in environmental samples, such as domestic sewage (Heuer *et al.* 2002; Tennstedt *et al.* 2003), hospital sewage (Reinthaler *et al.* 2003; Schwartz *et al.* 2003), sewage sludge (Guillaume *et al.* 2000; Reinthaler *et al.* 2003) coastal ponds (Meirelles-Pereira *et al.* 2002), underground waters (Gallert *et al.* 2005) and sewage-contaminated river waters (Costanzo *et al.* 2005).

The occurrence of strongly selective environments, such as hospitals, where large quantities of these drugs are used to prevent and treat infections, leads to an increase of multiresistant bacteria frequency that are released in hospital sewage (Meirelles-Pereira *et al.* 2002). Although many resistant bacteria have been isolated from environmental samples, there are only limited studies that have addressed the detection of extended-spectrum β-lactamase (ESBL)-producing bacteria from these types of samples (Mesa *et al.* 2006).

The emergence of multiresistance in the *Enterobacteriaceae* family, especially in *Klebsiella pneumoniae*, is a question that needs attention, because these are important causative agents of hospital infections, typically associated with pneumonias, blood stream infections, urinary tract infections, bacteremia and other intra-abdominal infections (Kotapati *et al.* 2005; Lopes *et al.* 2005; Grover *et al.* 2006; Sun *et al.* 2006). Additionally, studies have demonstrated the ESBL-producing bacteria are isolated with increasing frequency in many parts of the world (Peña *et al.* 1997; Araque *et al.* 2000; Seid and Asrat 2005; Grover *et al.* 2006; Kolar *et al.* 2006; Lee *et al.* 2006; Paterson 2006; Yu *et al.* 2006).

The aim of this study was to detect ESBL-producing *K. pneumoniae* in samples from the effluents and sludge of a hospital sewage treatment plant. Total and faecal coliforms were also determined in order to evaluate the efficiency of the plant in removing these micro-organisms.

Materials and methods

Characterization of the sewage treatment plant and sampling

The sewage treatment plant services a hospital located in the metropolitan area of the city of Rio de Janeiro (RJ), Brazil.

The treated sewage comes from the hospital's units, including laboratories, rehabilitation, dialysis, hospitalization, and surgery units, clinics, laundry and the cafeteria. The hospital serves an average of 2000 patients on a monthly basis, and has 800 employees working on a daily basis.

The hospital sewage treatment plant uses an anaerobic process (upflow anaerobic sludge blanket – UASB reactor), with a hydraulic retention time of 8 h, followed by post-treatment by three anaerobic filters arranged in a series, with a fill support made of crushed stone number four, with a hydraulic retention time of 4 h. In the pretreatment stage, the plant has a screen for the removal of gross solids. Mean influent flow measured is 2·54 l s^{-1}.

During the study, 20 sewage and sludge samples were collected in the period from May to December 2006. Five samples (500 ml) were collected on each day from the following: (i) influent (crude sewage); (ii) UASB reactor effluent; (iii) final filtered effluent; (iv) sludge taken from the UASB. All samples were transported to the laboratory refrigerated for immediate analysis.

Determination of environmental parameters

The physicochemical and microbiological parameters evaluated were pH, BOD_5 (biochemical oxygen demand over 5 days), COD (chemical oxygen demand), as well as total and faecal coliforms. The methodology used to assess the physicochemical parameters was consistent with the methods described in the *Standard Methods for Examination of Water and Wastewater* (APHA 1998). Samples were analysed for fecal coliforms according to the five-tube Most Probable Number (MPN) method (APHA 1998).

Isolation and Identification of *Klebsiella pneumoniae*

A volume of 10 μl from each sample and 10 μl of the following serial dilutions (10^{-1}, 10^{-2} and 10^{-3}) in saline was inoculated onto the culture media. The culture media used were tergitol- 7 (Oxoid, Basingstoke, Hampshire, England) agar with TTC (0·05% triphenyltetrazolium chloride), and EMB (eosin methylene blue agar) (Oxoid). After inoculation, the plates were incubated at 37°C for 24 h.

The mucoids and smooth colonies suggesting *K. pneumoniae* strains were inoculated onto the screening media for biochemistry identification: TSI (triple sugar iron), SIM (sulfate/indole/motility) and citrate agar, and incubated at 37°C for 24 h. Further biochemical testing to identify the species was performed as per the guidelines in the *Manual of Clinical Microbiology* (Murray *et al.* 2003).

Antimicrobial susceptibility tests

The *K. pneumoniae* strains isolated were submitted to antimicrobial susceptibility testing according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI 2007). The turbidity of the suspensions used for sensitivity testing was adjusted to 0·5 McFarland standard and inoculated onto Mueller-Hinton agar medium. The following antimicrobial discs (Oxoid) were used: ceftriaxone (CRO) (30 μg), ceftazidime (CAZ) (30 μg), cefepime (FEP) (30 μg), gentamicin (GEN) (10 μg), amikacin (AK) (30 μg), ciprofloxacin (CIP) (5 μg), chloramphenicol (C) (30 μg), trimethoprime/sulfamethoxazole (SXT) (1·25/23·75 μg) and tetracycline (TE) (30 μg). For quality control, the ATCC standard strains *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27953) and *Staphylococcus aureus* (ATCC 25923) were used.

Isolates that exhibited a zone of inhibition of growth for ceftazidime and ceftriaxone ≤22 mm and 25 mm, respectively, were submitted to the combined disc test in order to check for ESBL-producing strains.

ESBL detection

The combined disc methodology used to detect ESBL-producing *K. pneumoniae* was performed in agreement with the recommendations of the CLSI (2007). The

Table 1 Mean physicochemical and microbiological parameters obtained on each sampling points from the sewage treatment plant

Parameters	Influent medium/SD	UASB effluent medium/SD	Filtered effluent medium/SD
pH	6·9/0·11	6·9/0·13	6·6/0·47
BOD_5 (mg l^{-1})	29·6/12·6	10·8/4·5	4·6/4·5
COD (mg l^{-1})	161·4/42·7	67·7/14·5	27·7/16
Total coliforms (MPN 100 ml^{-1})	$1{\cdot}12 \times 10^8/7{\cdot}1 \times 10^{10}$	$2{\cdot}3 \times 10^8/8{\cdot}2 \times 10^8$	$2{\cdot}8 \times 10^6/7{\cdot}9 \times 10^6$
Faecal coliforms (MPN 100 ml^{-1})	$2 \times 10^7/9{\cdot}8 \times 10^8$	$6{\cdot}3 \times 10^6/1 \times 10^7$	$3{\cdot}5 \times 10^5/2{\cdot}1 \times 10^5$

BOD, biochemical oxygen demand; COD, chemical oxygen demand; SD, standard deviation; MPN, most probable number; UASB, upflow anaerobic sludge blanket.

antimicrobials (Oxoid) used were: cefotaxime (30 μg) and cefotaxime (30 μg) plus clavulanic acid (10 μg), and ceftazidime (30 μg) and ceftazidime (30 μg) plus clavulanic acid (10 μg). For quality control, the standard *K. pneumoniae* strain (ATCC 700603) was used.

Results were interpreted according to the criteria established by the CLSI (2007). A 5 mm increase in a zone of inhibition of growth for cefotaxime plus clavulanic acid as compared with the zone around the cefotaxime disc, and a 5 mm increase in the zone diameter for ceftazidime plus clavulanic acid as compared with the zone formed by the ceftazidime disc, were confirmatory for the result of ESBL-producing strains.

Results

Environmental parameters

The results from the physicochemical parameters (pH, BOD_5 and COD) can be found in Table 1. The pH values were within the expected range for values found in wastewater treatment plants that use an anaerobic process, and the BOD_5 and COD values were below the values commonly found in domestic sewage effluents. High concentrations of total coliforms were detected in the influent: $1{\cdot}12 \times 10^8 \pm 7{\cdot}1 \times 10^{10}$ MPN 100 ml^{-1}, and $2 \times 10^7 \pm 9{\cdot}8 \times 10^8$ MPN 100 ml^{-1} for faecal coliforms. The plant did not show a good efficacy in removing the coliforms, and high micro-organism concentrations were detected in the UASB reactor effluent, with $2{\cdot}3 \times 10^8 \pm 8{\cdot}2 \times 10^8$ MPN 100 ml^{-1} of total coliforms and $6{\cdot}3 \times 10^6 \pm 1 \times 10^7$ MPN 100 ml^{-1} of faecal coliforms. Even after treatment by the anaerobic filters, high concentrations of these micro-organisms were detected in the treated effluent, with $2{\cdot}8 \times 10^6 \pm 7{\cdot}9 \times 10^6$ MPN 100 ml^{-1} of total coliforms and $3{\cdot}5 \times 10^5 \pm 2{\cdot}1 \times 10^5$ MPN 100 ml^{-1} of faecal coliforms (Table 1).

Antimicrobial susceptibility tests

A total of 43 strains of *K. pneumoniae* were isolated from the effluents and the sludge of a sewage treatment process. ESBL-producing strains (20/43) were found in all the samples analysed (Table 2).

Table 2 Frequency (%) of extended-spectrum β-lactamase (ESBL)-producing *Klebsiella pneumoniae* isolates from the different stages of the hospital sewage treatment plant

Site (*n* = number of bacterial isolates)	ESBL-positive isolates *n* (%)	ESBL-negative isolates *n* (%)
Influent (*n* = 15)	8 (53·3)	7 (46·7)
Sludge (*n* = 6)	3 (50·0)	3 (50)
UASB effluent (*n* = 16)	7 (43·7)	9 (56·3)
Filtered effluent (*n* = 6)	2 (33·3)	4 (66·7)
Total (*n* = 43)	20 (46·5)	23 (53·5)

UASB, upflow anaerobic sludge blanket.

The ESBL-producing strains isolated also showed resistance to other antimicrobials: 70% were resistant to gentamicin, 35% to trimethoprime/sulfamethoxazole and tetracycline, 15% to amikacin and 10% to ciprofloxacin and chloramphenicol (Table 3). The 23 ESBL-negative strains showed less resistance to the antimicrobials tested, with 21·7% resistant to gentamicin, 17·3% to ceftriaxone and to trimethoprime/sulfamethoxazole, 13% to tetracycline and 8·6% to amikacin. The majority of the strains (95·7%) were susceptible to ceftazidime, cefepime, ciprofloxacin and chloramphenicol (Table 3).

Strains that expressed a phenotype of resistance to two or more classes of antimicrobials were regarded as multiresistant. In this way, all ESBL-producing strains were also considered multiresistant. Among the non-ESBL-producing strains, six (26%) demonstrated multiresistance (Table 3).

Multiresistant strains were detected in all the sewage samples analysed, and one strain, isolated from the treated effluent, was resistant to eight antimicrobials (Table 4).

Discussion

The introduction of sewage treatment plants that use an anaerobic process, such as an UASB reactor, has been increasingly encouraged in Brazil (Foresti 2001). These

Table 3 Antimicrobial susceptibilities of *Klebsiella pneumoniae* isolates

	ESBL-negative (n = 23) isolates		ESBL-positive (n = 20) isolates	
Antimicrobials	Resistance n (%)	Susceptibility n (%)	Resistance n (%)	Susceptibility n (%)
CRO	4 (17·3)	19 (82·7)	20 (100)	0 (0)
CAZ	1 (4·3)	22 (95·7)	20 (100)	0 (0)
FEP	1 (4·3)	22 (95·7)	20 (100)	0 (0)
GEN	5 (21·7)	18 (78·3)	14 (70)	6 (30)
AK	2 (8·6)	21 (91·4)	3 (15)	17 (85)
CIP	1 (4·3)	22 (95·7)	2 (10)	18 (90)
SXT	4 (17·3)	19 (82·7)	7 (35)	13 (65)
C	1 (4·3)	22 (95·7)	2 (10)	18 (90)
TE	3 (13)	20 (87)	7 (35)	13 (65)
MR*	6 (26)		20 (100)	

CRO, ceftriaxone; CAZ, ceftazidime; FEP, cefepime; GEN, gentamicin; AK, amikacin; CIP, ciprofloxacin; SXT, trimethoprime/sulfamethoxazole; C, chloramphenicol; TE, tetracycline; MR, multiple resistance; ESBL, extended-spectrum β-lactamase.

*Strains that were resistant to two or more antimicrobials were regarded as multiresistant.

plants have a relative advantage concerning the operational, construction and maintenance costs. In addition, it operates well at high temperatures, which is appropriate to tropical climate countries (Van Haandel and Lettinga 1994). Post-treatment by three anaerobic filters is not commonly reported. Therefore, it is important that the efficiency of this process be validated, principally in order to evaluate the capacity of the removal of pathogens.

The sewage treatment plant studied did not perform well in removing pathogenic micro-organisms. Although total and thermotolerant coliform concentrations were reduced in the final effluent by two log units as compared with the crude sewage concentrations, a high number of total and thermotolerant coliforms were detected in the filtered effluent, representing a risk for microbiological pollution of water resources. This point should be considered in the use of similar plants.

The presence of ESBL-producing *K. pneunoniae* in all analysed samples, showed the real possibility of bacteria circulation in the wastewaters coming from hospitals as well as in the sludge from the sewage treatment process of these establishments.

One of the recurring concerns because of the presence of ESBL-producing bacteria in these environments is associated with the transfer of conjugative plasmids, which also carry genes of resistance to aminoglucosides and sulfonamides, giving the bacteria multiresistance patterns (Paterson 2006). Conjugative plasmids can be easily transferred to other bacteria (Heuer *et al.* 2002; Tennstedt *et al.* 2003). This mechanism of gene transfer is particularly important in hospital effluents, because in such environments, the possibility of gene exchange is increased as a result of higher density and greater contact between these micro-organisms, which allows for an increased rate of horizontal transfer of these genes.

The incorrect handling and disposal of the effluents and the sludge of hospital sewage treatment plants into inappropriate locations may compromise public health by contaminating the soil, water supplies or recreational waters, and facilitate dissemination of micro-organisms and resistance genes into the environment.

It was demonstrated that the phenotypes found in the samples collected from the sewage treatment plant may

Table 4 Distribution of multiple resistances of ESBL-producing and non-ESBL- producing strains according to the number of antimicrobials tested

Sampling site	Influent		Sludge		UASB effluent		Filtered effluent	
Number of resistances*	Positive ESBL n	Negative ESBL n	Positive ESBL n	Negative ESBL n	Positive ESBL n	Negative ESBL n	Positive ESBL n	Negative ESBL n
Number of antimicrobials								
0	0	1	0	2	0	6	0	3
1	0	5	0	0	0	0	0	0
2	0	0	0	1	0	3	0	0
3	2	1	0	0	1	0	0	0
4	2	0	2	0	2	0	1	0
5	2	0	0	0	2	0	0	0
6	1	0	1	0	1	0	1	0
7	0	0	0	0	1	0	0	0
8	1	0	0	0	0	0	0	1
9	0	0	0	0	0	0	0	0

*Number of isolates which were resistant against zero to nine antimicrobials.

ESBL, extended-spectrum β-lactamase; UASB, upflow anaerobic sludge blanket.

be representative of the phenotypes circulating in the intrahospital environment (Guillaume *et al.* 2000). In this way, a high prevalence of resistant micro-organisms in hospitals shows a potential risk for disseminating pathogenic agents into the environment, particularly through the wastewaters.

In conclusion, although the efficiency for the removal of the organic burden by this system is not discussed, it was demonstrated in this study that the elimination of pathogenic micro-organisms failed. It is suggested that other post-treatment processes or the application of final disinfection processes for wastewaters be assessed for their capacity to reduce the microbial burden and be implemented with the intention of minimizing the impact from the release of hospital wastewaters into water resources, preventing dissemination of multiresistant micro-organisms and their genes of resistance into the environment, thus promoting prevention measures to protect public health.

References

American Public Health Association – APHA. (1998) *Standard Methods for the Examination Water and Wastewater*, 20th edn. Washington, DC: American Public Health Association.

Araque, M., Nieves, B., Lauretti, L. and Rossolini, G.M. (2000) Molecular basis of extended-spectrum β-lactamase production in nosocomial isolates of *Klebsiella pneumoniae* from Mérida, Venezuela. *Int J Antimicrob Agents* **15**, 37–42.

Clinical and Laboratory Standards Institute (CLSI). (2007) *Performance Standards for Antimicrobial Susceptibility Testing; Seventeenth Information Supplement*, 17th edn. Approved standard M100-S17. Wayne, PA: Clinical and Laboratory Standards Institute.

Costanzo, S.D., Murby, J. and Bates, J. (2005) Ecosystem response to antibiotics entering the aquatic environmental. *Mar Pollut Bull* **51**, 218–223.

Foresti, E. (2001) *Anaerobic Treatment of Domestic Sewage: Established Technologies and Perspectives*. In Proceedings of the 9th World Congress on Anaerobic Digestion – Anaerobic Conversion for Sustainability, 2–6 September. Antwerp, Belgium,

Gallert, C., Fund, K.L. and Winter, J. (2005) Antibiotic resistance of bacteria in raw and biologically treated sewage and in groundwater below leaking sewers. *Appl Microbiol Biotechnol* **69**, 106–112.

Grover, S.S., Sharma, M., Chattopadhya, D., Kapoor, H., Pasha, S.T. and Singh, G. (2006) Phenotypic and genotypic detection of ESBL mediated cephalosporin resistance in *Klebsiella pneumoniae*: emergence of high resistance against cefepime, the fourth generation cephalosporin. *J Infect* **53**, 279–288.

Guillaume, G., Verbrugge, D., Chasseur-Libotte, M.L., Moens, W. and Collard, J.M. (2000) PCR typing of tetracycline resistance determinants (Tet A–E) in *Salmonella enterica* serotype Hadar and in the microbial community of activated sludges from hospital and urban wastewater treatment facilities in Belgium. *FEMS Microbiol Ecol* **32**, 77–85.

Heuer, H., Krogerrecklenfort, E., Wellington, E.M.H., Egan, S., Van Elsas, J.D., Van Overbeek, L., Collard, J.M., Guillaume, G. *et al.* (2002) Gentamicin resistance genes in environmental bacteria: prevalence and transfer. *FEMS Microbiol Ecol* **42**, 289–302.

Kolar, M., Latal, T., Cermak, P., Bartonikova, N., Chmelarova, E., Sauer, P. and Kesselova, M. (2006) Prevalence of extended-spectrum β-lactamase-positive *Klebsiella pneumoniae* isolates in the Czech Republic. *Int J Antimicrob Agents* **28**, 49–53.

Kotapati, S., Kuti, J.L. and Nightingale, C.H. (2005) Clinical implications of extended spectrum β-lactamase (ESBL) producing *Klebsiella* species and *Escherichia coli* on cefepime effectiveness. *J Infect* **51**, 211–217.

Kummerer, K.L. (2001) Drugs in the environment: emission of drugs. Diagnostic aids and disinfectants into wastewater by hospitals in relation to other sources – a review. *Chemosphere* **45**, 957–969.

Lee, H., Yong, D., Yum, J.H., Roh, K.L.H., Lee, K.L., Yamane, K.L., Arakawa, Y. and Chong, Y. (2006) Dissemination of 16S rRNA methylas-mediated highly amikacin-resistant of *Klebsiella pneumoniae* and *Acinetobacter baumannii* in Korea. *Diagn Microbiol Infect Dis* **56**, 305–312.

Lopes, A.C.S., Rodrigues, J.F. and Morais Júnior, M.A. (2005) Molecular typing of *Klebsiella pneumoniae* isolates from public hospitals in Recife, Brazil. *Microbiol Res* **160**, 37–46.

Meirelles-Pereira, F., Pereira, A.M.S., Silva, M.C.G., Gonçalves, V.D., Brum, P.R., Castro, E.A.R., Pereira, A.A. and Pereira, J.A.A. (2002) Ecological aspects of the antimicrobial resistance in bacteria of importance to human infections. *Braz J Microbiol* **33**, 287–293.

Mesa, R.J., Blanc, V., Blanch, A.R., Cortés, P., González, J.J., Lavilla, S., Miró, E., Muniesa, M. *et al.* (2006) Extended-spectrum-β-lacatamase-producing *Enterobacteriaceae* in different environments (humans, food, animal farms and sewage). *J Antimicrob Chemother* **58**, 211–215.

Murray, P.R., Baron, E.J., Jorgensen, J.H., Pfaller, M.A. and Yolken, R.H. (2003) *Manual of Clinical Microbiology*, 8th edn Washington, DC: ASM Press.

Paterson, D.L. (2006) Resistance in gram-negative bacteria: *Enterobacteriaceae*. *Am J Med* **119**, S20–S28.

Peña, C., Pujol, M., Ricart, A., Ardanuy, C., Ayats, J., Linãres, J., Garrigosa, F., Ariza, J. *et al.* (1997) Risk factors for faecal carriage of *Klebsiella pneumoniae* producing extended spectrum β-lactamase (ESBL – KP) in the intensive care unit. *J Hosp Infect* **35**, 9–16.

Reinthaler, F.F., Posch, J., Feierl, G., Wust, G., Hass, D., Ruckenbauer, G., Mascher, F. and Marth, E. (2003) Antibiotic

resistance of *E.coli* in sewage and sludge. *Water Res* **37**, 1685–1690.

Schwartz, T., Kohnen, W., Jansen, B. and Obst, U. (2003) Detection of antibiotic-resistant bacteria and their resistance genes in wastewater, surface water, and drinking water biofilms. *FEMS Microbiol Ecol* **43**, 325–335.

Seid, J. and Asrat, D. (2005) Occurrence of extended spectrum β-lactamase enzymes in clinical isolates of *Klebsiella* species from Harar region, eastern Ethiopia. *Acta Trop* **95**, 143–148.

Sun, H.Y., Chen, S.Y., Chang, S.C., Pan, S.C., Su, C.P. and Chen, Y.C. (2006) Community-onset *Escherichia coli* and *Klebsiella pneumoniae* bacteremia: influence of health care exposure on antimicrobial susceptibility. *Diagn Microbiol Infect Dis* **55**, 135–141.

Tennstedt, T., Szczepanowski, R., Braun, S., Pühler, A and Schlüter, A. (2003) Occurrence of integron-associated resistance gene cassetes located on antibiotic resistance plasmids isolated from a wastewater treatment plant. *FEMS Microbiol Ecol* **45**, 239–252.

Van Haandel, A.C. and Lettinga, G. (1994) *Anaerobic Sewage Treatment: A Practical Guide for Regions with a Hot Climate*. New York, USA: John Wiley and Sons.

Yu, Y., Ji, S., Chen, Y., Zhou, W., Wei, Z., Li, L. and Ma, Y. (2006) Resistance of strains producing extended-spectrum β-lactamases and genotype distribution in China. *J Infect* **54**, 53–57.