ANTIMICROBIAL RESISTANCE OF ENTERIC BACTERIA ISOLATED FROM A SEWAGE TREATMENT PLANT

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RESUMO

O presente trabalho analisou a freqüência de bactérias em uma estação de tratamento de esgoto em Porto Alegre (RS, sul do Brasil). Amostras de água foram coletadas cada três meses, no período de julho/1997 a junho/1998, do afluente, na lagoa de estabilização, na lagoa de maturação e no efluente da estação. Também foram coletadas amostras de dois locais fora da estação (71a e 71b). A resistência a antimicrobianos foi determinada pelo teste de difusão em ágar Muller-Hinton e 18 antimicrobianos diferentes foram testados. Entre as 839 cepas isoladas, *Enterobacter* sp (n=348) e *Escherichia coli* (n=210) foram as mais observadas, seguido por *Proteus* sp (n=66), *Serratia* (n=53), *Citrobacter* (n=40), *Klebsiella* (n=37), *Yersinia* sp (n=32), *Shigella* (n=28) e *Salmonella* (n=25). Uma redução no número de bactérias totais e cepas resistentes a antimicrobianos (cr), pode ser observada do afluente ($4{,}69 \times 10^4$ufc/mL; 154 cr) ao efluente ($7{,}94 \times 10^3$ ufc/mL; 49 cr) da estação de tratamento. O número de bactérias resistentes nos pontos 71a e 71b foram tão altos quanto no afluente. O número de cepas multiresistentes também reduziu significativamente do afluente para o efluente. O presente estudo demonstrou que o sistema de tratamento de esgoto estudado é eficiente na redução de enterobactérias e da quantidade de bactérias resistentes a antimicrobianos, contribuindo para um menor lançamento de bactérias multiresistentes no ambiente.

Palavras chave: enterobactérias, esgoto, resistência antimicrobiana, ambiente aquático

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ABSTRACT

The present work analysed the frequency of antimicrobial resistant enteric bacteria in a sewage treatment plant in Porto Alegre (RS, Southern Brazil). Water samples were collected every three months, from July/1997 to June /1998, at the inflow, at the facultative stabilization lagoon, at the maturation lagoon and the effluent of the plant. Two sites outside the plant (71a and 71b) were also sampled. Antimicrobial resistance was determined by disc diffusion test on Mueller-Hinton agar, where 18 different antimicrobials were tested. Among the 839 isolated strains, *Enterobacter* sp (n=348) and *Escherichia coli* (n=210) were the most frequently observed ones, followed by *Proteus* sp (n=66), *Serratia* sp (n=53), *Citrobacter* sp (n=40), *Klebsiella* sp (n=37), *Yersinia* sp (n=32), *Shigella* sp (n=28) and *Salmonella* sp (n=25). The total number of bacteria and antimicrobial resistant strains (rs) decreased from inflow ($4.69x10^4$cfu/mL;154 rs) to effluent ($7.94x10^3$ cfu/mL;49 rs) at the treatment plant. At sites 7a and 7b the number of resistant strains was as high as the inflow. The number of multiresistant strains also reduced significantly from the inflow to the effluent. The present study demonstrates that the sewage treatment system investigated is efficient in decreasing enterobacteria and antimicrobial resistant bacterial numbers, contributing to a lower discharge of multiresistant bacteria into the environment.

Key words: enterobacteria, sewage, antimicrobial resistance, aquatic environment

INTRODUCTION

At the present time, antimicrobial resistant bacteria can be found in all environments, all aquatic ecosystems and in all climatic zones. The incidence of multiresistant bacteria in aquatic environment has increased during the last years, resulting in a serious environmental and health problem since many commensal organisms, like some enteric bacteria, harbour various resistance genes (KRUSE, 1994; WIGGINS, 1999; COSTA et al., 2006; AUERBACH et al., 2007). A selective pressure in favour of bacteria possessing these genes has emerged from the abusive use of antimicrobial drugs mainly in hospitals, agriculture and animal farming (GOLD, 1996; DAVIES, 1997; MULAMATTATHIL, 2000). Liquid manure of animals as well as human excretions has also led to dissemination of resistant enteric bacteria in the environment (REINTHALER, 2003). There is some speculation about levels of antimicrobial drugs that have not been degraded or eliminated during sewage treatment process, which would be contributing to this selective pressure within aquatic environments (FORD, 1997).

The genetic flexibility of bacteria has contributed to their survival in altered environments, because of their capacity to acquire and transfer resistance genes. Gene transfer has been observed in aquatic environments (GEALT, 1985; MORINIGO, 1990; ANGLES, 1993), and has led various researchers to concentrate their studies on distribution and survival of resistant bacteria and how they contribute to this increase in antimicrobial resistance levels.

Before the abusive antimicrobial use age, only a slight resistance level had been observed among enteric bacterium species. Nowadays, their susceptibility to antimicrobials has changed, and resistant patterns have been used as epidemiologic markers. They are frequently associated with infectious diseases and they are among the bacterium species that have been used to investigate environmental resistance patterns (FILALI, 2000; GOÑI-URIZA, 2000) and many are also used as indicators of faecal pollution in natural waters (WHITLOCK , 2002; REINTHALER, 2003).

Bacterial populations may adjust themselves to many adversities found in the aquatic environment (available nutrients, temperature, pH, microbial predation, parasitism and antagonism) by decreasing growth rates and surviving in this hostile environment. Many of them pose no risks to water supplies, although others can be pathogenic and opportunistics, causing taste, odour and even health problems when the water they

thrive in is consumed. Appropriate treatment has to be carried out to inactivate or decrease these bacteria numbers, thus avoiding the formation of biofilms in sand filters, the spreading of multiresistance and colonization in distribution systems (GELDREICH, 1996).

The present study was designed to analyse the frequency of antimicrobial resistant enteric bacteria along a sewage treatment process. With this purpose, sample collection took place at four different points inside and outside of a sewage treatment plant in Porto Alegre city, Southern Brazil. Enteric bacteria were isolated from these samples and their antimicrobial resistance patterns analysed.

MATERIAL AND METHODS

ANALYSED AREA AND SAMPLE COLLECTION

The samples were collected at Ipanema Sewage Treatment Plant, in Porto Alegre city, Southern Brazil, every four months from July/1997 to June/1998. This treatment plant has a system of stabilization lagoons that consists of three pairs of treatment lagoons: anaerobic (7,960.0 m², with an incoming waste of about 23,880.0 m³), facultative (30,464.0 m², with an incoming waste of about 45,696.0 m³) and maturation (17,711.6 m² with an incoming waste of about 26,567.5 m³). The whole treatment system has an area of 24.4 ha; a total volume of incoming waste of about 194,971.0 m³ and the total retention time in the plant is 9.17 days.

The first sampling site was at the inflow of the plant, the second one was at the facultative stabilisation lagoon; the third, at the maturation lagoon; the fourth, at the effluent of the plant (at Salso stream); the fifth, at Salso stream (71a), approximately 200 m upstream the effluent and the sixth was at the end of Salso stream (71b), approximately 200 m downstream the effluent at the stream, close to Guaiba Estuary.

ISOLATION AND IDENTIFICATION OF BACTERIAL STRAINS

In 500-ml sterilised bottles, 250 ml of water were collected at a depth of 60 cm from the surface and then stored at 4°C until processed at the laboratory, within 4 h after sampling. The samples were diluted from 10^{-1} to 10^{-3} in peptone water and 0.1 mL of the dilutions were plated in triplicate onto selective media, Eosin Methilene Blue agar (EMB, BIOBRAS), Yersinia agar (BIOBRAS), and onto plate counting agar (PCA, Merck). The selective media plates were incubated at 37°C and the PCA, at 30°C for 48 hours. For the *Salmonella* and *Shigella* identification, one mL of each sample was inoculated on 9mL of peptone water, and after 24 hours at 37°C, 0.1 ml of this was seeded on XLT4 (MERCK) and XLD (MERCK) triplicate agar and incubated at 37°C for 24 hours.

Colonies were chosen from definite sectors of the plate (approximately 10% of the total count) from the selective agar. The same number of colonies from each selective agar was picked for each sampling site. For pure isolates, colonies were seeded by multiple streaking on the respective selective agar. After checking their purity, the isolated colony was seeded on Trypticase Soy Agar (BIOBRAS). Each of the colonies was identified according to the response to the following biochemical tests: oxidation-fermentation, carbohydrate fermentation, production of enzymes (oxidase, catalase, urease, amino acids decarboxilases), production of indol and H_2S, use of citrate, Voges-Proskauer , Methyl-Red, always using negative and positive ATCC (American Type Culture Collection) control strains. All the biochemical tests were done according to MacFaddin, 1996, and identification of the strains was based on Bergey's Manual (HOLT, 1994). Although the same number of colonies was initially chosen for each sampling site, at the end different colony numbers were available because many of the colonies failed to respond to biochemical tests. A collection of the pure identified colonies was prepared on Brain Heart Infusion/15% glycerol.

ANTIMICROBIAL SUSCEPTIBILITY TESTING

The methodology used to assess antimicrobial susceptibility was based on disc-diffusion method. MacFarland standard 0.5 (1.5×10^8 ufc/ml) was used as inoculum. The following antimicrobial drugs (CEFAR) were tested: amoxicillin (30ug), ampicillin (30ug), carbenicillin, oxacillin (10ug), penicillin G (10ug), cephalothin (30ug), cephoxithin (30ug), nalidixic acid (30ug), norfloxacin (10ug), streptomycin (10ug), gentamicin (10ug), tetracycline (30ug), chloramphenicol (30ug), erythromycin (15ug), sulfonamides (30ug), trimetropin (25ug), riphampicin (30ug) and nitrofurantoin (30ug), were placed on Mueller Hinton agar plates (MERCK). The strains were classified as sensitive, intermediate or resistant based on the inhibition zone diameter, according to the National Committee for Clinical Laboratory Standards guidelines (NCCLS, 2000). All strains showing "resistant" or "intermediate" behaviour were subsumed under the category "resistant", while the others were classified as "sensitive".

STATISTICAL ANALYSIS

For the statistical evaluation, chi-square tests and the Friedman test were used to compare differences in

mean values. All these tests were performed in a SAS 8.0 program.

Results

Along the sewage treatment plant a significant decrease in the number of colonies was observed, from $4.69x10^4$ cfu/mL at the inflow to $7.94x10^3$ cfu/mL at the effluent ($p<0.01$). Sites 71a and 71b, with $5.57\ x10^4$ and $5.34\ x10^4$ cfu/mL respectively, showed significantly higher numbers ($p<0.01$) than the inflow (Table 1). A total of 839 enteric bacteria were isolated from the selective agar plates and they could be identified to at least the genus level: 348 *Enterobacter*, 210 *Escherichia*, 66 *Proteus*, 53 *Serratia*, 40 *Citrobacter*, 37 *Klebsiella*, 32 *Yersinia*, 28 *Shigella* and 25 *Salmonella* (Table 2). No significant difference was observed within the distribution of the genera along the sewage treatment plant, although higher numbers of isolates were observed among the ones from the inflow, when comparing with the sites within de treatment process. These strains were analysed with regard to their antimicrobial susceptibility.

For most of the identified genus the number of resistant strains decreased significantly, from the inflow to the effluent ($p=0.024$), with exception of *Citrobacter*, *Yersinia* and *Salmonella* strains (Table 3). *Escherichia* and *Enterobacter* strains were resistant to most of the β-lactams (amoxicillin, ampicillin, oxacillin and penicillinG), cephems (cephalotin and cephoxithin) and sulfonamides in all collecting sites of the treatment plant. A decrease in the number of resistant strains was observed in these genera for carbenicillin, chloramphenicol and tetracycline, from around 50% at the inflow to less than 10% at the other sites. *Proteus*, *Serratia*, *Citrobacter*, *Klebsiella*, *Yersinia*, *Shigella* and *Salmonella* had similar numbers of resistant strains to β-lactams, cephems, sulfonamides, chloramphenicol and tetracycline, however *Klebsiella* strains were resistant to chloramphenicol (60 % at facultative lagoon and 50% at maturation lagoon) and *Shigella* to tetracycline (60 % at facultative lagoon and 50% at maturation lagoon). For other antimicrobials the percentages varied between genera, but susceptibility to the quinolones and aminoglycosides was common to all of them.

Although a high percentage of resistant strains was observed for 15 out of the 19 analysed antimicrobials, most of them showed a significant decrease in these percentages from inflow to the effluent of the treatment plant ($p<0.05$), with the exception of penicillinG, cephalotin and erythromycin , which had higher percentages of resistance at the facultative lagoon (Table 4). There was a less significant decrease when comparing inflow with facultative lagoon, and effluent with maturation lagoon. The percentages at the outside sampling sites 71a and 71b were significantly higher ($p<0.05$). Among the lactams, carbenicillin showed the lowest percentage of resistant strains. In all sampling sites a low percentage of resistant strains was observed for quinolones (nalidixic acid and norfloxacin), and aminoglycosides (gentamicin and kanamycin).

The multiresistance pattern along the sewage treatment plant was only verified between *Enterobacter* and *Escherichia* strains because of their sample size in the different sampling sites. Bacteria resistant to at least two antimicrobials were considered as multiresistant. The multiresistance pattern varied from 2 to 16 antimicrobials at the effluent of the treatment plant and from 2 to 9, at the effluent (Table 5). A reduction in the number of multiresistant bacteria could be observed along the collecting sites. A strong association ($p<0.01$) between inflow and multiresistance to 12/13 antimicrobials; facultative and maturation and multiresistance to 8/9 antimicrobials; effluent and multiresistance to 4/5 antimicrobials, was observed. Sampling sites 71a and 71b showed association to multiresistance from 10 to13 antimicrobials. There was a significant difference between inflow and effluent ($p<0.05$). The most common multiresistance pattern observed was to 11 antimicrobials at the effluent, 9 at facultative lagoon, 7 at maturation lagoon and 5 at the effluent of the treatment plant.

Discussion

Presence of antimicrobial resistant bacteria (polluted and non-polluted) has been observed in different aquatic environments (KELCH and LEE, 1978; BOON, 1992; RICE *et al*., 1995; GOÑI-URIZA *et al*., 2000; MCARTHUR and TUCKFIELD, 2000; HARDWOOD *et al*., 2000; COSTA et al., 2006;). Sewage treatment plants are known to reduce organic matter of effluents and also to remove faecal and total coliform (GUARDABASSI *et al*, 2002; REINTHALER *et al*., 2003). By analysing the results obtained at Ipanema Sewage Treatment plant, a reduction in the total number of bacteria, in enteric bacterial isolates and antimicrobial resistant bacterial isolates was observed within the treatment plant, demonstrating the efficiency of the treatment in removing enterobacteria. This contributed to a lower discharge of resistant enterobacteria into streams and consequently to a decrease in antimicrobial resistant bacteria released back into the aquatic environment.

Although the number of strains varied between genera, for most of genera a decrease within the treatment plant was observed pointing to the efficiency of the treatment in removing enterobacteria. *Enterobacter* strains were the most frequent; with numbers that were reduced at the facultative and maturation lagoons but increasing at the effluent. *E. coli*, a faecal pollution indicator and the second most frequent isolate, reduced in 43% within the treatment plant (Table 1). Mezrioui and Baleux (1994) found a reduction of around 99% of faecal

coliforms in an aerobic lagoon system, which is different from the treatment system analysed in the present study. Lim and Flint (1989) observed that *E. coli* is capable of growing in sewage. However, if other competitive bacteria are present, there is a rapid decrease in its number of viable cells. This may explain why *E. coli* numbers were lower than *Enterobacter* in this study. In the present study, sewage treatment also resulted in a reduction of organic matter (data not shown), which could contribute to a reduction in the presence of pathogenic bacteria.

The efficiency of the treatment in removing resistant enterobacteria was observed in five of the nine analysed genera. Four of them did not show a reduction in the number of resistant isolates (*Proteus, Citrobacter, Yersinia* and *Salmonella*); this might be due to the low number of strains per sampling site. Mckeon et al.(1995) who studied antimicrobial resistance in Gram negative bacteria in rural groundwater supplies, found a higher percentage of resistant bacteria among noncoliforms than coliforms; this was also observed in the present study, although noncoliforms (*Proteus*, *Serratia, Citrobacter*, *Yersinia*, *Shigella* and *Salmonella*) were in low numbers. Mezrioui and Baleux (1994) observed a higher percentage of antimicrobial resistant *E. coli* isolates in domestic sewage after treatment in an aerobic lagoon and this is probably due to the selection of resistant strains by this kind of treatment, where antimicrobial resistance would be favouring bacterial survival in this environment. Guardabassi et al (2002), analysing the effects of a tertiary treatment on the prevalence of antimicrobial resistant bacteria, concluded that the isolates from treated sewage and digested sludge were not significantly more resistant compared with isolates from raw sewage. They concluded that the analysed treatment did not result in a selection of antimicrobial resistant bacteria. High percentages of resistant isolates were observed among the bacteria isolated from site 71a, outside the sewage treatment plant. This also demonstrates the efficiency of the treatment in reducing the numbers of resistant strains and not contributing to their selection, since these water samples did not suffer any kind of treatment.

At the sewage treatment plant analysis, low frequency of quinolone and aminoglycoside resistant strains were observed at all sites. McKeon et al. (1995) and Goñi-Urriza et al. (2000) had also reported low percentages of resistance to these antimicrobial groups among enterobacteria; the first ones were isolated from rural ground water, and the second, from a river upstream and downstream a wastewater discharge. Reinthaler et al. (2003) observed that *E. coli* strains isolated from sewage treatment plants were less resistant against quinolones. Gram-negative bacteria are known to be susceptible to quinolones and these synthetic antimicrobials are naturally absent in aquatic environments.

The multiresistance also decreased within the sewage treatment plant, from the pattern of 11 antimicrobials at the effluent, to the pattern of 5 antimicrobials at the effluent. These findings are not in agreement with Mezrioui and Baleux (1994), who indicate an increase in multiresistance among *E.coli* isolated from the inflow and outflow of an aerobic lagoon; they concluded that resistance could be acquired during the sewage treatment. Plasmids carry most of the antimicrobial resistance and their maintenance can be expensive to bacteria. Once there is a low level of organic matter in the environment, bacteria would naturally eliminate them, mainly if these plasmids are not essential to their survival, in order to save energy. Therefore, reduction of organic matter within the sewage treatment plant, contributes not only to a reduction in the number of bacteria, but also to a reduction in antimicrobial resistant bacteria.

Although this study demonstrates that the analysed sewage treatment system is efficient in decreasing antimicrobial resistant enterobacteria numbers, it also demonstrates that multiresistant enterobacteria are still reaching the receiving water (71b) and contributing to the dissemination of multiresistance in the environment. Multiresistant bacteria are a major public health concern due to the emergence of various pathogenic bacteria resistant to most antimicrobial agents available for human therapy. On the other hand, we must keep in mind that this water will suffer a reduction of organic matter content and chemical treatment before entering the water distribution system, and that it will also contribute to the reduction of enterobacteria numbers and consequently multiresistant strains, reaching a better condition for human use.

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Table 1 – Distribution of the total bacteria count within the sewage treatment plant

Sampling sites	Total bacteria count (CFU/ mL)		
	Med.	Min	Max
Inflow	$4.69x10^4$	$4,65x10^4$	$4,74x10^4$
Facultative lagoon	$2.39x10^4$	$2,37x10^4$	$2,41x10^4$
Maturation lagoon	$1.72x10^4$	$1,60x10^4$	$1,78x10^4$
Effluent	$7.94x10^3$	$7,74x10^4$	$8,05x10^4$
71a	$5.57x10^4$	$5,19x10^4$	$5,87x10^4$
71b	$5.34x10^4$	$4,95x10^4$	$5,48x10^4$

Table 2 – Distribution of the isolated enterobacteria within the sewage treatment plant

Enterobacteria isolated within the sewage treatment plant	Number of bacteria isolates						
	IN	FAC	MAT	EF	71a	71B	Total
Enterobacter spp	64	59	45	56	59	65	348
Escherichia coli	51	21	30	22	48	38	210
Proteus spp	18	06	04	01	18	19	66
Serratia spp	11	10	11	06	08	07	53
Citrobacter spp	11	05	05	03	06	10	40
Klebsiella spp	06	05	04	04	09	09	37
Yersinia spp	11	04	03	03	03	08	32
Shigella spp	08	05	04	04	03	04	28
Salmonella spp	13	03	03	02	01	03	25
Total	193	118	109	101	155	163	839

IN – inflow; FAC – facultative lagoon; MAT – maturation lagoon; EF – effluent; 71a – sampling site 71a; 71b – sampling site 71b)

Table 3 - Percentage of the resistant enterobacteria isolated along the treatment plant

Enterobacteria isolates	Sampling sites					
	IN	FAC	MAT	EF	71a	71b
Enterobacter spp	93.75 (60/64)	93.22 (55/59)	62.22 (28/45)	39.28 (22/56)	71.18 (42/59)	70.76 (46/65)
Escherichia coli	96.07 (49/51)	100.00 (21/21)	63.33 (19/31)	59.09 (13/22)	75.00 (36/48)	78.94 (30/38)
Proteus spp	100.00 (18/18)	83.33 (05/06)	75.00 (03/04)	100.00 (01/01)	94.40 (17/18)	100.00 (19/19)
Serratia spp	90.90 (10/11)	81.81 (09/10)	72.72 (08/11)	66.66 (04/06)	75.00 (06/08)	85.71 (06/07)
Citrobacter spp	90.90 (10/11)	80.00 (04/05)	80.00 (04/05)	100.00 (03/03)	66.66 (04/06)	80.00 (08/10)
Klebsiella spp	83,00 (05/06)	100.00 (05/05)	75.00 (03/04)	50.00 (02/04)	66.66 (06/09)	88.88 (08/09)
Yersinia spp	90.90 (10/11)	100.00 (04/04)	100.00 (03/03)	66.66 (02/03)	100.00 (03/03)	87.50 (07/08)
Shigella spp	87.50 (07/08)	80.00 (04/05)	75.00 (03/04)	50.00 (02/04)	100.00 (03/03)	75.00 (03/04)
Salmonella spp	92.30 (12/13)	100.00 (03/03)	66.66 (02/03)	100.00 (02/02)	100.00 (01/01)	100.00 (03/03)
Mean of resistance ± sd	91.70 ± 4.85	90.93 ± 9.45	74.44 ± 11.30	70.19 ± 23.90	83.21 ± 14.99	85.19 ± 10.22

IN – inflow; FAC – facultative lagoon; MAT – maturation lagoon; EF – effluent; 71a – sampling site 71a; 71b – sampling site 71b

Number in parenthesis indicated number of resistant bacteria per total number in each sampling site

Table 4. Percentage of resistant bacteria for each antimicrobial drug within the sewage treatment plant

	Sampling sites					
Antibiotics Tested	IN n= 193	FAC n= 118	MAT n= 109	EF n= 101	71a n= 155	71b n= 163
Oxa*	89.64	83.89	71.43	50.49	71.61	73.61
Ery	89.12	92.37	61.47	45.54	54.84	53.37
Cep	84.97	86.44	56.88	39.60	54.83	57.05
Rif	82.38	77.12	52.29	29.70	47.09	52.76
PenG	75.65	78.81	59.63	40.59	58.71	63.19
Nit	77.20	70.34	44.95	38.61	59.35	61.35
Cfo	72.02	69.49	39.45	31.68	57.42	59.51
Amo	73.57	65.25	41.28	31.68	47.74	45.39
Amp	66.32	73.73	44.95	33.66	44.51	45.39
Sul	65.28	56.78	32.11	21.78	40.64	46.63
Tmp	57.51	47.46	30.28	23.76	60.65	60.12
Tet	53.88	31.36	12.84	9.90	26.45	33.12
Clo	46.63	43.22	23.85	20.79	48.39	42.33
Str	43.01	27.97	23.85	20.79	34.19	33.13
Car	40.93	37.29	18.35	17.82	37.42	43.56
Kan	33.16	18.64	9.17	6.93	19.35	24.54
Gen	32.12	16.10	11.93	5.94	19.35	22.70
Nal	6.73	8.47	3.67	1.98	8.39	13.49
Nor	5.18	5.93	1.83	0.99	3.87	4.29
Mean ± sd	57.65 ± 25.71	52.14 ± 28.12	33.69 ± 21.04	24.85 ± 14.95	41.83 ± 18.87	43.97 ± 18.21

IN – inflow; FAC – facultative lagoon; MAT – maturation lagoon; EF – effluent; 71a – sampling site 71a; 71b – sampling site 71b), n - number of isolates for each sampling site

*Oxa–oxacillin; Ery–erythromycin; Cep–cephalothin; Rif–rifampicin; PenG-penicillin G; Nit-nitrofurantoin; Cfo-cefoxithin; Amo-amoxicillin; Amp-ampicillin; Sul-sulfonamides; Tmp-trimetropin; Tet-tetracycline; Clo-chloramphenicol; Str-streptomycin; Car-carbenicillin; Kan-kanamycin; Gen-gentamicin; Nal-nalidixic acid; Nor-norfloxacin