Occurrence and resistance of pathogenic bacteria along the Tietê river downstream of São Paulo in Brazil

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Abstract

The load of pathogenic bacteria, their fate and their dangerousness in the Tietê river were assessed along 100 km starting from the city of São Paulo in Brazil and compared with bacteria of two German rivers. High load of pathogens were found in the Tietê river in the city of São Paulo (*E. coli* O157:H7, *Shigella flexneri* and *S. boydii*), which were absent 30 km downstream of São Paulo. The antibiotic resistances observed in the Tietê river were rather low and decreased after the major input in São Paulo to significantly lower levels about 30 km downstream. While the Brazilian isolates were more susceptible for ampicillin than the German ones the reverse was observed for gentamycin. For optimal control of infections in humans critical areas where these bacteria survived longer and their elimination mechanisms should be identified as well as the extent and the origin of antibiotic resistance.

1. Introduction

For the city of São Paulo in Brazil the Tietê river is an important water reservoir, however, especially in the city of São Paulo with its estimated 25 million inhabitants its water is heavily loaded with untreated waste of all types and it is assumed that the sewage of about 10 million persons is washed without any treatment into the river. Due to the high demand for water in the city of São Paulo the water of Tietê river is repeatedly fed into water works before it leaves the city. Such an intensive use of the water resources requires careful monitoring of the water quality to exclude risks for human health. The water of Tietê river is routinely controlled by CETESB and the bulk parameters are determined at currently 154 stations [1]. The water is here monitored by the indexes for raw water for public supply, protection of aquatic life, phytoplankton community, zooplankton community of reservoirs, bentonic community that involves 50 physical, chemical, hydrobiological, microbiological (thermotolerant coliforms, *Crypstoporidium sp.* and *Giardia* sp.) and ecotoxicological parameters. There are also 34 parameters for sediment analysis but none of these parameters were exclusively directed to pathogenic or facultative pathogenic bacteria. To help closing this gap this study was focused on this aspect and the results compared with the data available from CETESB. The sampling sites were close to monitoring sites of CETESB and for the sampling date the rainy season in December was chosen where sewage from households together with other organic waste is continuously washed into the river.

It is also important to elucidate what the fate of these bacteria was and how fast they were cleared after leaving the city of São Paulo. To do this the quantification of colony forming units (cfus) on agars recommended for the quantification of different pathogens became essential. Although, the media used in this study do not only select for pathogenic but also for related bacteria, which occur in the environment as well but are not pathogenic, their viable cell counts are an important parameter in such studies [2]. The results can be applied for a more detailed study directed to identify critical areas where these bacteria survived longer and could cause a potential thread and actions against them could be undertaken. The knowledge on such reservoirs of pathogenic bacteria enables very dedicated actions and, therefore, the prevention of possible dangers to humans.

A study on the potential danger caused by bacteria introduced during the severe flood of the Elbe river in August 2002 found a surprisingly high antibiotic resistance of isolates from flooded cellars [3]. The origin of this resistance could not be unambiguously determined but it was assumed that extensive application of antibiotic in agriculture could be a reason for the high resistance observed. Similar suggestions have been made for the increasing antibiotic resistance in clinical isolates [4]. In this context we were interested to determine how isolates from different locations of Tietê river could handle antibiotics and we wanted to compare the results with those from Germany. For the comparison we chose the Elbe river as one of the main German streams passing through several industrialised areas in the Czech Republic and Germany [5]. Additionally, we took a sample from the Oker river as one of the minor rivers in Germany not polluted by industrial activities [6]. Because both countries have rather different applications of antibiotics both in medicine and in agriculture it was expected that such a comparison could give insights into the extent and the origin of antibiotic resistance.

2. Material and Methods

2.1. Sampling

The Tietê river was sampled in the rainy season on December 9, 2003 along a section of about 100 km at four stations between São Paulo and the city of Salto to monitor the survival of pathogenic bacteria and their antibiotic resistance. Sampling site 1 was located between the monitoring stations TIET 04200 ($23^{0}31^{'}33^{''}$S/$46^{0}44^{'}47^{''}$W) and TAMT04900 ($23^{0}23^{'}38^{''}$S/$46^{0}59^{'}46^{''}$W) and site 2 was close to the station TIPI 04900 (Figure 1). The Oker river and the Elbe river in Germany were sampled for comparison on October 6, 2003. The sampling sites were determined by GPS with a position accuracy of <15 m and are listed in Table 1. Per site 100 ml of water were collected from the surface of the river and stored in two sterile tubes (50 ml per tube). The samples dedicated for isolation on the different media were plated immediately after the return to the laboratory.

2.2. Selective enrichments

Colony forming units (cfu) were determined on different selective media using incremental dilution of the water samples. The media were *Salmonella Shigella* agar (SS agar) (selective for *Escherichia*, *Enterobacter*, *Salmonella*, *Shigella*, *Proteus*), Gassner agar (selective for *Escherichia* and *Staphylococcus*), bile aesculin agar (selective for *Enterococcus* and *Streptococcus*) and Endo agar (selective for *Escherichia*, *Enterobacter*, *Klebsiella*). The Tietê river samples were also grown on MacConkey agar (selective for *Enterobacteriaceae*) [7]. All agar plates were cultivated at 30°C for 48 h according to the EU recommendations for water hygiene [8] and the colonies were counted separately according to the agar used.

2.3. Identification of isolates

From the selective agars of Tietê river water single colonies were randomly selected and isolated by restreaking the colony on plates to ensure that uniform colonies were obtained. From site 1 the isolates WAB1888 – WAB1917 (AM184229-AM184258), WAB1919 – WAB1922 (AM184259-AM184262) and WAB1924 - WAB1929 (AM184263-AM184268), from site 2 WAB1945 – WAB1961 (AM184284-AM184300) and WAB1963 - WAB1969 (AM1842301-AM184307), from site 3 WAB1867 - WAB1884 (AM1842209-AM194226) and WAB1886 – WAB 1887 (AM184227-AM184228) and from site 4 WAB1930 - WAB1944 (AM184269-AM184283) were obtained (GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of the strains are given in brackets). The isolates were phylogenetically identified by sequencing their 16S rRNA genes and comparison of the sequences with those in public and in-house databases. Near complete 16S rRNA gene sequences from the strains listed in Table 3 were amplified by PCR and sequenced as described previously [9]. The reactions were evaluated on an Applied Biosystems 377 genetic analyser. The program SEQUENCHER™ Version 4.0.5 (Gene Codes Corporation, USA) was used to analyse the sequences. The sequence was matched in BLAST 2.2.9 [10] against the EMBL database [11]. The sequences were aligned using Clustal X software [12] and the phylogenetic analysis was performed using MEGA 3.1. software [13].

2.4. Tests for antibiotic resistances

Individual isolates were plated on LB medium and exposed to 10 µg ampicillin, 15 µg erythromycin, 10 µg gentamycin or 30 µg vancomycin on individual paper disks [14]. Additionally, 36 isolates were tested against 30 µg kanamycin, 30 µg novobiocin or 10 µg bacitracin. Isolates were termed resistant if the zone of inhibition around the antibiotic disks were smaller than 13 mm for the antibiotics ampicillin and erythromycin, 12 mm for gentamycin, 9 mm for vancomycin, 13-17 mm for kanamycin, 17-21 mm for novobiocin and 8-12 mm for bacitracin and controlled after 24, 48 and 72 h [15]. If the inhibition zone were larger the isolates were termed sensitive and if the inhibition zone was exactly this value they were regarded as being at the borderline between sensitive and resistant and termed ambiguous according to the recommendations of the manufacturer. Thirty-six additional isolates from the Tietê river were additionally tested for their sensitivity against the antibiotics kanamycin, novobiocin and bacitracin.

3. Results

The numbers of colony forming units (cfu) were determined for all but MacConkey agar plates for each site, the MacConkey agar was only used for the Brazilian samples (Figure 2). Because of their significant differences the two samples from the Marginal Tietê site in the city of São Paulo were shown both here. The viable cell counts are the highest in São Paulo and in the city of Pirapora do Bom Jesus. They declined rapidly and reached a level of <3000 cfu ml^{-1} at sites 3 and 4. A slight increase can be seen between samples from sites 3 and 4, which may reflect the influence of the city of Salto on Tietê river. The viable cell counts from the Oker river in Germany were in the same range than those from sites 3 and 4 of Tietê river, but those of the Elbe river were even lower.

A comparison of the viable cell counts from the different selective media revealed than the bile esculin agar showed the highest number of colonies. This was found for all sampling sites in both countries for all rivers. The second highest colony numbers were found on Gassner agar but only for the sites with the highest cell counts, i. e. the Marginal Tietê and Pirapora do Bom Jesus sites. The sites with intermediate cell counts, i. e. sites 3 and 4 in Brazil and the Oker and Elbe river samples did not follow this trend but had higher viable cell counts on Endo or *Shigella Salmonella* (SS) agar.

From the agar plates of the Tietê river samples 102 isolates have been obtained and identified by comparison of their 16S rRNA gene sequences with those from public databases. A large number of different genera were identified but some tendencies could be seen between the samples. Only from the Marginal Tietê site situated in São Paulo city *Escherichia coli* strains could be isolated. It is very remarkable that three of these isolates showed 99% similarity on the basis of the 16S rRNA genes to the highly pathogenic *E. coli* strain O157:H7 also known as EHEC [16, 17]. Furthermore, two strains closely related to *Shigella boydii* were found. Ten *Enterobacter* isolates, six *Aeromonas* of which three belonged to *Aeromonas hydrophila*, one to *A. caviae*, and one *Klebsiella pneumoniae* were obtained from the Marginal Tietê site in São Paulo and gave a clear indication of the high load of pathogenic bacteria at this site. From the water sampled from Pirapora do Bom Jesus four *Enterobacter* species and seven *Aeromonas* strains, four of them *A. hydrophila* were isolated. With *Shigella flexneri* one of the three *Shigella* species found in this study was isolated from this site. From the site 3, situated between Pirapora do Bom Jesus and Salto five *Aeromonas* strains were isolated, two of them closely related with *Aeromonas hydrophila* and one with *A. veronii*. Furthermore, two *Enterobacter* species were identified. Finally, site 4 brought only one *Aeromonas hydrophila* isolate. All sites had a number of different *Pseudomonas* species and, remarkably, *Pseudomonas plecoglossicida*, a fish pathogen [18], was found at the Marginal Tietê site.

The isolates from the Tietê river samples were tested on their sensitivity against four different antibiotics and the results compared with those obtained for isolates from the rivers Oker and Elbe [3]. Since only 17 isolates from the Elbe river were characterised the comparison concentrated on the Oker isolates where 29 strains were available. The sensitivities of the strains isolated on the different selective media against four antibiotics are shown in Figure 3.

The isolates from the Tietê river could be killed mainly by using ampicillin or gentamycin. According to the different preferences of the bacteria for the media the antibiotic resistance of the strains differed with their source of isolation. While ampicillin was most efficient for isolates from Gassner agar gentamycin was more successful for isolates originating from bile esculin or SS agar. Erythromycin could only control isolates from bile esculin agar but even here only 13% of the strains were killed. Vancomycin is a special case here because this antibiotic effects the biosynthesis of the cell wall of Gram-positive bacteria [19]. As a consequence the highest sensitivity towards this antibiotic was found for the bile esculin agar isolates. Beside some of the few Gram-positive isolates four Gram-negative strains were found to be sensitive to vancomycin. These strains were identified as closely related to species of *Acinetobacter*, *Aeromonas, Comamonas* and *Enterobacter*. Summarising the sensitivity for all strains showed that gentamycin was the most effective antibiotic closely followed by ampicillin. Both erythromycin and vancomycin were almost useless in the control of the isolates (Figure 3). Checking the isolates for multiresistance identified 26% of them to be resistant or ambiguous in the antibiotic tests. It is worthwhile to note here that all *E. coli* O157:H7 isolates, the two *Shigella boydii* strains and *Shigella flexneri* could be killed with ampicillin.

A comparison of the antibiotic resistance of the strains according to their origin did not give a clear tendency for the Tietê river samples (Figure 4). The sensitivity for ampicillin was between 33% and 50% of the strains from a given site, where the lowest sensitivity came from site 3 and the highest from the Marginal Tietê site. Their was a weak tendency of an increase in the gentamycin sensitivity from the Marginal Tietê site (30%) to sites further downstream where the Pirapora do Bom Jesus site had the highest value (72%) and Salto site the second highest (60%). For erythromycin and vancomycin the activities were too rare to be comparable. To increase the database 36 isolates were additionally tested against kanamycin, novobiocin and bacitracin. Almost all strains tested were sensitive against kanamycin, only two isolates from Marginal Tietê showed resistance against this antibiotic. The opposite was the case for novobiocin where only one isolate from site 3 was sensitive. Most isolates were resistant against bacitracin and only 5 sensitive strains were detected. When the mean resistance of the isolates was determined it was found that each isolates from Salto displayed resistances against 3.57±0.98 antibiotics, those from site 3 against 3.22±1.30, from Pirapora against 4.33±1.03 and the Marginal Tietê isolates against 4.75±1.04 and 4.67±1.03 antibiotics of the seven antibiotics tested.

The isolates from the German Oker river (and the Elbe river as well) gave a somewhat different picture. Here the best antibiotic to control the bacteria was gentamycin, which killed at least 70% of the isolates (Figure 4). All isolates from the Gassner agar could be controlled by gentamycin. The next effective antibiotic was erythromycin but even the most sensitive strains, isolated from bile esculin agar could be killed by only 27% (Figure 3). Ampicillin inhibited less than 10% of the isolates but none of the Gassner and SS agar strains. As for the Tietê river isolates vancomycin was not very efficient and killed only 9%, all of them obtained from the bile esculin agar. The sum of the antibiotic resistance showed a high sensitivity of the strains against gentamycin, a much lower one against erythromycin and only small activities of ampicillin and vancomycin.

4. Discussion

The high levels of colony forming units on the media used reflect the influence of the megacity São Paulo. The input of bacteria into Tietê river decreased rather fast and the high viable cell counts in the river at Pirapora do Bom Jesus at a sampling site which is located after the Pirapora Dam probably reflects both the influence of São Paulo city and local input from the city of Pirapora do Bom Jesus (Figure 1). This was evident from the sampling site were a thick layer of oil and dirt was swimming on the river. CETESB reported for 2003 high organic loads for the stations TAMT 04900 and TIET 04200, flanking the Marginal Tietê site. The eutrophic situation is slightly lower at the Pirapora station TIPI 04900. This trend is also found in the reported number of thermotolerant coliforms (Table 2) [1]. Our results showed that the number of coliforms do not exactly reflects the pathogenic potential of the Tietê river water where three isolates of *E. coli* O157:H7 could be obtained from the Marginal Tietê site.

Further downstream from Pirapora do Bom Jesus the viable cell counts fell rapidly and the site 3, which was not close to a town, and the Salto site showed viable cell counts, which were close to those found in the German river Oker. Such a decrease in pathogens was also reported from an urban river in north-east Brazil [20]. However, the much larger river Elbe gave even less viable cell counts. The higher viable cell counts of the river Oker compared to the river Elbe are not so easily to explain. One reason may be the input from pastures which should be more pronounced at the river Oker because of its much smaller size than at the river Elbe. However, other sources of input remain possible. It should be noted that the viable cell counts determined for samples from flooded buildings after the severe Elbe flood in August 2002 was much higher than the data determined for any samples of the Tietê river [3]. This underlines that in the cellars and mud samples conditions different from rivers prevail.

The finding of *Shigella flexneri* in the Pirapora sample and *S. boydii* in the Marginal Tietê samples is remarkable because shigellosis is an increasing problem worldwide [21, 22]. The same arguments hold for the two *E. coli* O157:H7 isolates which are also severe pathogens [23]. However, the pathogenicity of the Marginal Tietê isolates has not been confirmed in an animal model. It should be noted here that the genus *Shigella* is very closely related to the genus *Escherichia* and it has been suggested that these two genera actually form only one genus [24, 25, 26]. Interestingly, the high numbers in *Aeromonas caviae* reported from the freshwater systems in Marrakech [27] were not found in the Tietê river where *A. hydrophila* prevailed.

Gentamycin was the most effective antibiotic for the strains of all but one site. For the Brazilian strains the second most important antibiotic was ampicillin. However, this is not the case for the German isolates, which were more sensitive against erythromycin than to ampicillin. Erythromycin had much less effect on the strains from Brazil than on those from Germany. For most of the tested antibiotics this tendency of efficiency did not vary much between the sites of a country but showed dramatic differences between the two countries. Especially ampicillin and erythromycin showed different effects on the strains from Brazil and Germany. The most probable explanation for this is a difference in the use of antibiotics in the two countries causing different resistance against certain antibiotics. An alternative interpretation may be a different resistome in the soil as has recently been reported by D'Costa *et al*. [28].

For several years the problem of increasing resistance of clinical isolates against antibiotics and the development of multiresistance has been discussed and the growing amount of evidences had led to a drastic reduction in the use of antibiotics in non-medical applications in some countries. Recent studies in these countries confirmed that the effect is reversible and the level of antibiotic resistance can be decreased if the application of antibiotics in agriculture is reduced as a report of the WHO has shown for Denmark [29]. Therefore, it can be assumed that the bacteria isolated from Tietê river and those from the two German rivers have different antibiotic resistance, especially against ampicillin, because the use of antibiotics in both countries is different. It is interesting to note that the multiresistance for antibiotics decreased from the city of São Paulo over Pirapora to Salto. While the decrease from the Marginal Tietê site in São Paulo to Pirapora do Bom Jesus is only small, the decrease to site 3 and Salto is substantial. Such a decrease may be explained by the massive loss of plasmids carrying the resistance genes in the bacteria [30]. The decrease in antibiotic resistance was found for both pathogens and non-pathogens excluding the possibility that the resistance was passed from poorly surviving pathogens to non-pathogens. A similar decrease in multiresistance has been reported for the Arga river, Spain, by Gońi-Urizza *et al*. [31].

In future better predictions how pathogenic bacteria will behave in the different habitats are required [32] and it is important to identify the sites, where they do not impose potential dangers and those where they should be controlled. It is generally assumed that these fecal bacteria do not survive long in the rivers because they are allochthonous in these habitats [33] and the results of this study support this. However, in order to control them it is necessary to determine how fast they are killed and what the elimination mechanisms are. Furthermore, the CETESB report showed a pronounced decrease of precipitation in the São Paulo area over the last 50 years, a tendency which will also have an impact on the numbers and the survival of pathogens in Tietê river.

Conclusion

Summarizing the results:

- High load of pathogens were found in the Tietê river in the city of São Paulo (*E. coli* O157:H7, *Shigella flexneri* and *S. boydii*).
- Low antibiotic resistances were observed in the Rio Tietê but the resistance profile was different to the one found in German rivers.
- The antibiotic resistance decreases after the major input in São Paulo to significantly lower levels about 30 km downstream.

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Table 1

Sampling sites

No.	Site	Location		Altitude
		Latitude	Longitude	[m]
Brazil: Tietê river				
1	São Paulo, Marginal Tietê	23°S 30.496'	46°W 41.809'	720
2	Pirapora do Bom Jesus	23°S 23.848'	47°W 00.214'	656
3	between Salto and Pirapora	n.d.[a]	n.d.	n.d.
4	Salto, Parque das Lavras	23°S 13.160'	47°W 17.482'	518
Germany: Oker river				
5	Hillerse	52°N 24.586’	10°E 23.892’	50
Germany: Elbe river				
6	Dömitz, Elbe km 503.2	53°N 13.67’	11°E 7.75’	12

[a]not determined

Table 2

Colony forming units (cfu ml^{-1}) determined for different selective media and sites and physico-chemical data for sites sampled by CETESP in 2003 which are adjacent to the sampling sites.

Site no.	Endo agar	Gassner agar	SS agar	Bile esculin agar	MacConkey agar
1-1	12160	16980	1370	19044	14080
1-2	4720	6240	160	16720	830
2	7080	8640	4680	23600	8800
3	1640	640	470	2840	380
4	3120	2200	150	3880	370
5	1067	367	1467	2667	-
6	320	60	233	633	-

Physico-chemical data of CETESP samples

Station	Conductivity	Turbidity	NO_2	NO_3	NH_3	OD	DBO	Residues	Surfactants	P total	Coliforme termophile [1]
TAMT04900	545	37	0.338	0.35	15.99	0.1	95	341	2.32	2.520	4700
TIET04200	586	40	0.015	0.23	15.03	0.1	46	274	2.48	2.178	2000
TIPI04900	545	24	0.148	0.51	12.88	0.1	32	278	2.62	2.095	1300
TIRG02900	531	19	0.076	0.42	16.13	0.8	21	250	2.39	1.575	420
TIET02350	507	29	0.468	1.44	14.92	6.5	24	343	1.03	1.364	530

[1]Cfu of CETESP converted to cfu/ml

Table 3

Identified isolates from Tietê river, their isolation medium and antibiotic susceptibilities. Ampicillin = AM, Erythromycin = ER, Gentamycin = GE, Vancomycin = VA and BP = Base Pairs; diameter of the inhibition zone around the antibiotic paper disk in millimeters (mm); - not susceptible, + susceptible; ± ambivalent; BA Bile Aeculin.

Isolate	**Medium**	AM	ER	GE	VA	**Closest match**	**Identity**
WAB 1888	Endo	-	-	+	-	*Comamonas terrigena*	98.4
WAB 1889	Endo	-	-	+	-	*Pseudomonas putida* or *P. plecoglossicida*	99.9
WAB 1890	Endo	+	-	±	-	*Shigella boydii* or *Escherichia coli*	99.6
WAB 1891	Endo	-	-	-	-	*Kluyvera ascorbata*	99.6
WAB 1892	Gassner	+	-	-	-	*Escherichia coli O157:H7*	99.8
WAB 1893	Gassner	+	-	-	-	*Shigella boydii* (very similar to *E. coli* O157:H7)	99.4
WAB 1894	Gassner	+	-	-	-	*Enterobacter ludwigii* or *Pantoea* sp.	99.6
WAB 1895	Gassner	±	-	-	-	*Kluyvera ascorbata*	99.5
WAB 1896	Gassner	+	-	-	-	*Enterobacter* sp.	99.2
WAB 1897	SS Agar	+	-	-	-	*Enterobacter* sp.	99.5
WAB 1898	SS Agar	-	-	±	-	*Pseudomonas putida* KT2440	99.9
WAB 1899	BA Agar	+	-	+	-	*Acinetobacter* sp.	98.7
WAB 1900	BA Agar	+	-	+	-	*Acinetobacter* sp.	98.0
WAB 1901	BA Agar	-	-	+	-	*Aeromonas hydrophila*	99.7
WAB 1902	BA Agar	-	-	-	-	*Enterobacter* sp.	99.5
WAB 1903	Mac Conk.	±	-	-	-	*Enterobacter* sp.	98.7
WAB 1904	Mac Conk.	±	-	-	-	*Kluyvera cryocrescens*	98.0
WAB 1905	Mac Conk.	-	-	+	-	*Aeromonas hydrophila*	99.8
WAB 1906	Mac Conk.	+	-	-	-	*Enterobacter aerogenes*	99.1
WAB 1907	Mac Conk.	-	-	-	-	*Enterobacter* sp.	99.5
WAB 1908	Endo	+	-	-	-	*Citrobacter freundii*	99.3
WAB 1909	Endo	-	-	-	-	*Klebsiella ornithinolytica*	99.6
WAB 1910	Endo	+	-	+	-	*Acinetobacter* sp.	99.2
WAB 1911	Endo	+	-	±	-	*E. coli* (very similar to *E coli* O157:H7)	99.8
WAB 1912	Endo	-	-	-	-	*Klebsiella pneumoniae*	99.5
WAB 1913	Gassner	+	-	+	-	*Pantoea agglomerans*	99.5

Isolate	Medium	AM	ER	GE	VA	Closest match	Identity
WAB 1914	Gassner	+	-	+	-	*Acinetobacter haemolyticus*	96.7
WAB 1915	Gassner	+	-	-	-	*Enterobacter* sp.	99.5
WAB 1916	SS Agar	-	-	+	-	*Aeromonas* sp.	98.9
WAB 1917	SS Agar	-	-	+	-	*Acinetobacter* sp.	95.8
WAB 1919	SS Agar	-	-	±	-	*Aeromonas* sp.	99.8
WAB 1920	SS Agar	-	-	+	-	*Aeromonas hydrophila*	99.7
WAB 1921	BA Agar	+	+	+	+	*Kurthia gibsonii*	96.8
WAB 1922	BA Agar	-	-	+	-	*Aeromonas hydrophila*	99.9
WAB 1924	BA Agar	+	-	-	-	*Pantoea agglomerans*	99.3
WAB 1925	Mac Conk.	+	-	+	-	*Pantoea agglomerans*	99.7
WAB 1926	Mac Conk.	-	-	+	-	*Enterobacter* sp.	99.5
WAB 1927	Mac Conk.	+	-	-	-	*Pantoea agglomerans*	98.7
WAB 1928	Mac Conk.	-	-	+	-	*Aeromonas caviae*	99.1
WAB 1929	Mac Conk.	+	-	±	-	*Enterobacter* sp.	99.5
WAB 1945	Endo	±	±	+	-	*Comamonas testosteroni*	99.5
WAB 1946	Endo	+	-	-	-	*Enterobacter ludwigii*	99.0
WAB 1947	Endo	-	-	+	-	*Pseudomonas putida* KT2440	99.8
WAB 1948	Endo	-	±	+	-	*Aeromonas hydrophila*	99.9
WAB 1949	Endo	-	-	±	-	*Pseudomonas putida* KT2440	99.9
WAB 1950	Gassner	+	-	+	-	*Acinetobacter* sp.	98.8
WAB 1951	Gassner	+	-	-	-	*Pantoea agglomerans*	99.0
WAB 1952	Gassner	+	-	+	-	*Acinetobacter johnsonii*	99.1
WAB 1953	SS Agar	-	-	+	-	*Aeromonas caviae*	99.5
WAB 1954	SS Agar	-	±	+	-	*Aeromonas caviae*	99.7
WAB 1955	SS Agar	-	-	+	-	*Aeromonas caviae*	99.5
WAB 1956	SS Agar	±	-	-	-	*Enterobacter* sp.	99.4
WAB 1957	SS Agar	-	-	+	-	*Aeromonas caviae*	99.6
WAB 1958	SS Agar	+	-	+	+	*Aeromonas* sp.	99.7
WAB 1959	SS Agar	+	±	+	-	*Enterobacter* sp.	98.7
WAB 1960	BA Agar	-	-	-	-	*Pseudomonas* sp.	99.7
WAB 1961	BA Agar	+	±	+	±	*Acinetobacter junii*	98.8
WAB 1963	BA Agar	-	-	+	-	*Pseudomonas* sp.	99.9
WAB 1964	BA Agar	+	±	+	-	*Comamonas* sp. D22	97.1
WAB 1965	BA Agar	+	+	+	+	*Comamonas* sp.	99.8
WAB 1966	Mac Conk.	+	-	+	-	*Shigella flexneri*	99.5
WAB 1967	Mac Conk.	-	-	-	+	*Enterobacter* sp.	99.1
WAB 1968	Mac Conk.	-	-	+	-	*Aeromonas hydrophila*	99.8
WAB 1969	Mac Conk.	+	-	-	-	*Pantoea agglomerans* or *Enterobacter aerogenes*	99.0

Isolate	Medium	AM	ER	GE	VA	Closest match	Identity
WAB 1867	Endo	+	±	+	+	*Acinetobacter* sp.	98.6
WAB 1868	Endo	-	-	+	-	*Aeromonas* sp.	99.9
WAB 1869	Endo	-	-	-	-	*Pseudomonas putida*	99.9
WAB 1870	Endo	+	-	-	-	*Pantoea agglomerans*	99.5
WAB 1871	Endo	±	±	-	-	*Comamonas testosteroni*	99.7
WAB 1872	Gassner	+	-	-	-	*Pantoea agglomerans*	98.9
WAB 1873	Gassner	-	-	±	-	*Pseudomonas mosselii*	99.5
WAB 1874	Gassner	-	-	-	-	*Comamonas testosteroni*	99.6
WAB 1875	SS Agar	-	-	+	-	*Aeromonas hydrophila*	99.8
WAB 1876	SS Agar	-	-	+	-	*Aeromonas* sp.	99.7
WAB 1877	SS Agar	-	-	+	-	*Aeromonas hydrophila*	99.7
WAB 1878	BA Agar	±	-	+	-	*Enterobacter cloacae*	99.8
WAB 1879	BA Agar	-	-	+	-	*Pseudomonas putida* KT2440	99.9
WAB 1880	BA Agar	+	+	+	-	*Comamonas* sp.	99.6
WAB 1881	BA Agar	-	-	±	-	*Pseudomonas putida*	99.2
WAB 1882	BA Agar	-	±	±	-	*Aeromonas veronii*	99.7
WAB 1883	BA Agar	+	-	+	+	*Bacillus* sp.	99.1
WAB 1884	Mac Conk.	+	-	-	-	*Enterobacter* sp.	99.5
WAB 1886	Mac Conk.	+	-	+	-	*Acinetobacter junii*	99.8
WAB 1887	Mac Conk.	-	-	±	-	*Pseudomonas fulva or P. parafulva*	99.8
WAB 1930	Endo	-	-	+	-	*Burkholderia cepacia*	98.6
WAB 1931	Endo	+	±	+	-	*Acinetobacter* sp.	98.7
WAB 1932	Endo	+	±	+	-	*Acinetobacter johnsonii*	98.0
WAB 1933	Gassner	±	-	-	-	*Pantoea agglomerans*	99.2
WAB 1934	Gassner	+	-	+	-	*Acinetobacter* sp.	98.5
WAB 1935	SS Agar	-	-	+	-	*Pseudomonas putida* KT2440	99.9
WAB 1936	SS Agar	+	-	±	-	*Enterobacter* sp.	98.4
WAB 1937	SS Agar	±	-	-	-	*Serratia* sp.	96.0
WAB 1938	BA Agar	-	-	-	-	*Enterobacter* sp.	97.4
WAB 1939	BA Agar	+	±	+	-	*Acinetobacter johnsonii*	99.2
WAB 1940	BA Agar	±	-	+	-	*Acinetobacter junii*	97.4
WAB 1941	BA Agar	+	±	+	-	*Acinetobacter* sp.	98.6
WAB 1942	Mac Conk.	+	-	-	-	*Citrobacter freundii*	99.5
WAB 1943	Mac Conk.	-	-	±	-	*Aeromonas hydrophila*	99.8
WAB 1944	Mac Conk.	-	-	+	-	*Burkholderia caryophylli*	99.7

Figure 1. Site map

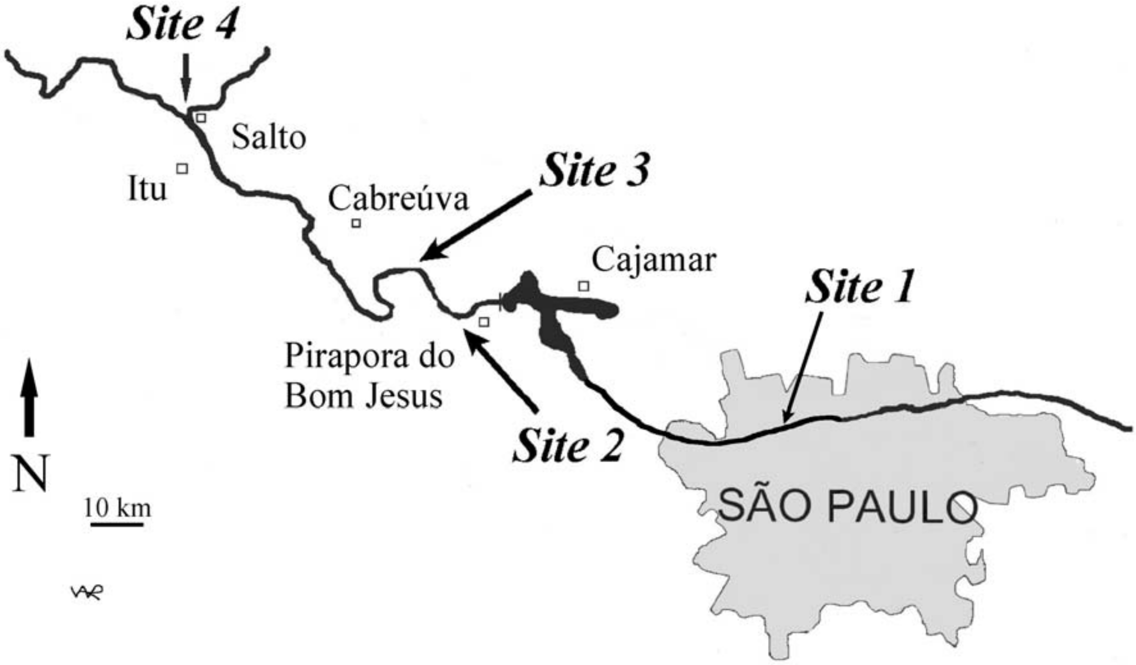


Figure 2. Colony forming units (cfu) determined for the different selective media and sites**.** Black: Endo agar; gray: Gassner; diagonal strips: SS; horizontal points: Bile esculin; light gray: MacConkey agar (not determined for sites 5 and 6).

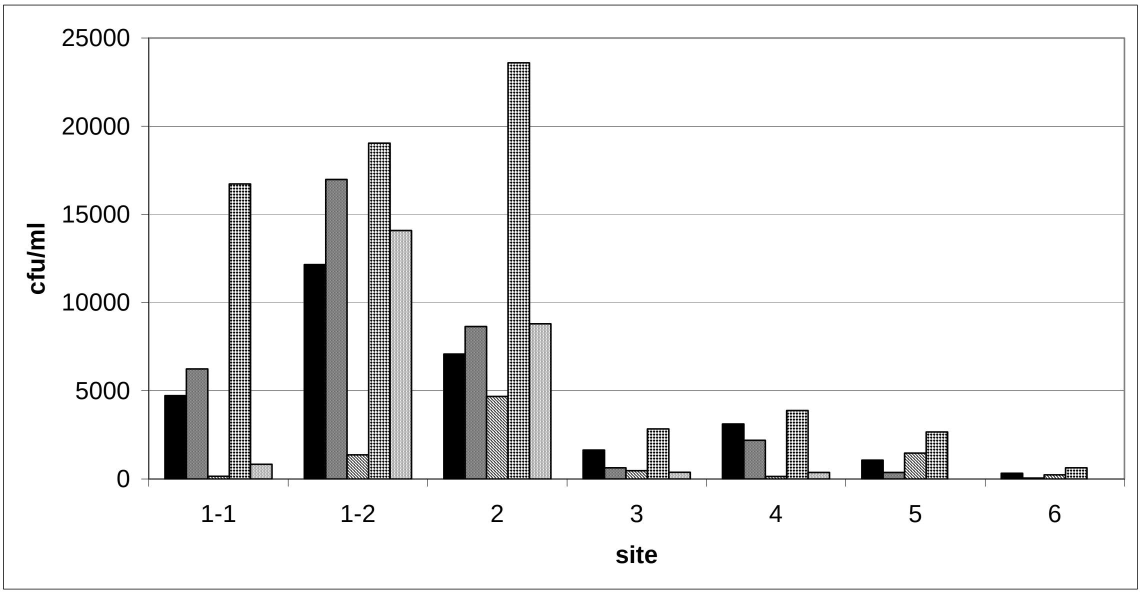
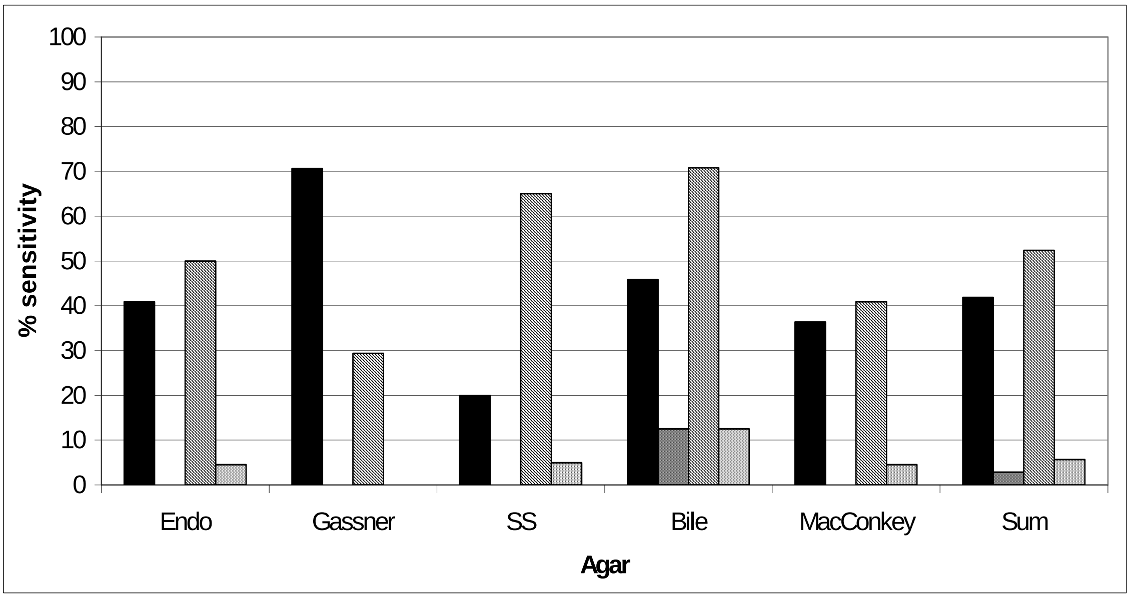


Figure 3. Susceptibilities against the antibiotics ampicillin (black bars), erythromycin (grey bars), gentamycin (diagonally striped bars) and vancomycin (light grey bars) of strains isolated from Tietê river and Oker river on five different media. The strains from the Oker river were not isolated on MacConkey agar, therefore, the columns were left open. To the right of the diagrams the sensitivities of all isolates against the antibiotics were summed up.

Tietê river



Oker river

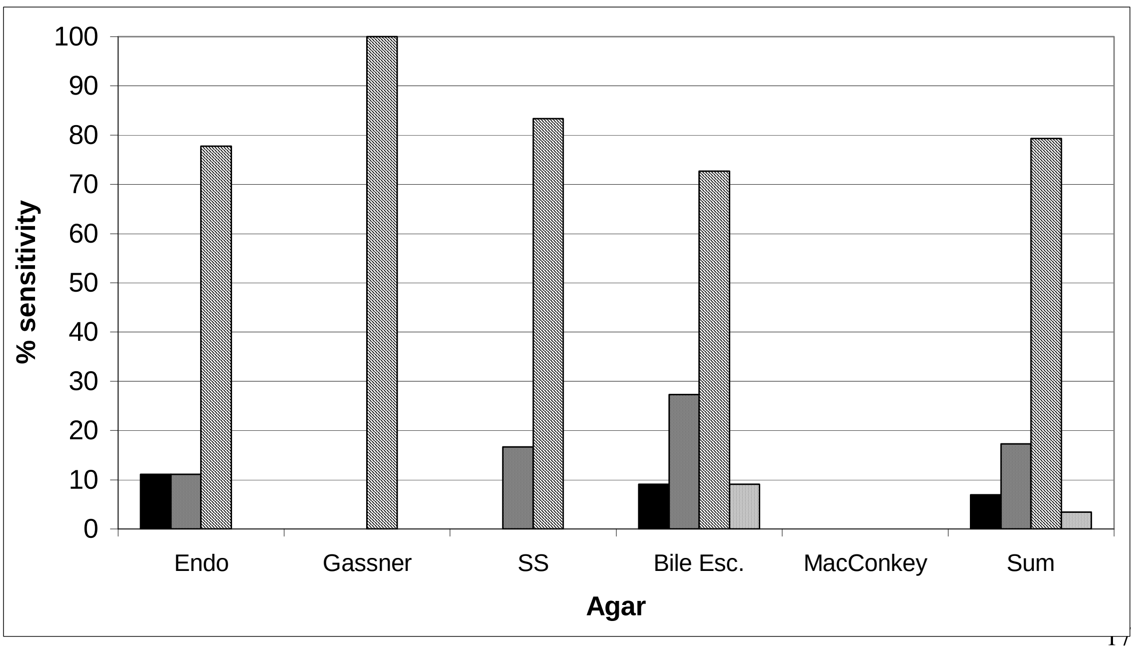
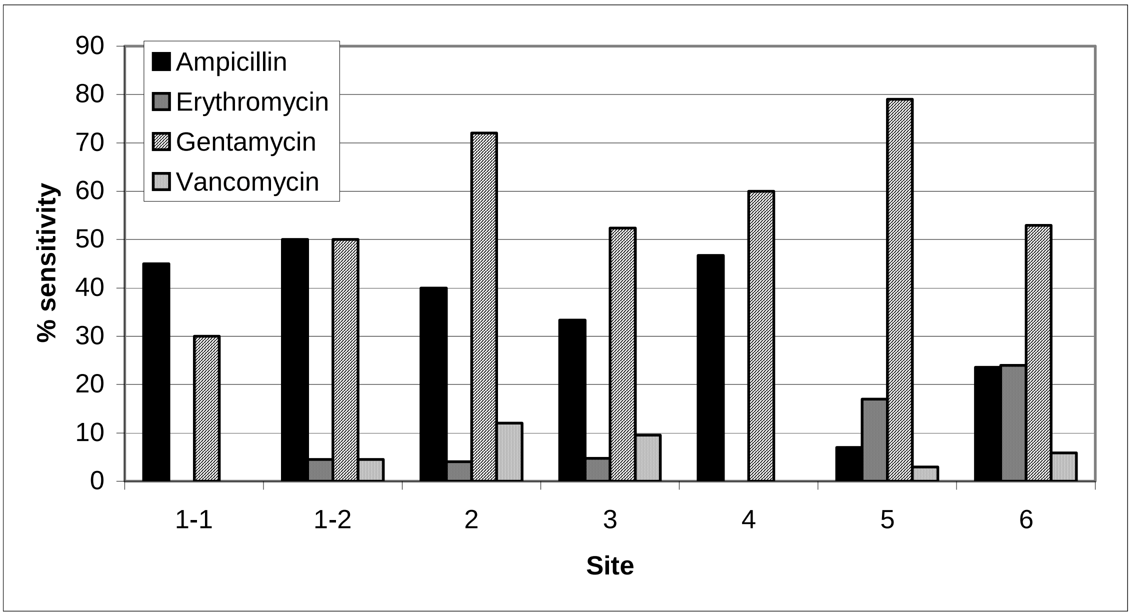


Figure 4. Antibiotic resistance determined for isolates from the different sampling sites



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