



Full Length Article

Resistance Variations of Third Generation of Cephalosporins in Some of the Enterobacteriaceae Members in Hospital Sewage

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ABSTRACT

Bacterial resistance to commonly used antibiotics is monitored for a long time by researchers around the world. To estimate the resistance variation to 3rd generation Cephalosporin antibiotics, total 1457 strains of the *Enterobacteriaceae* family (*Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella pneumonia*) were isolated from hospital sewage water. Resistance rates were determined against CRO, ZOX and CTX within two different periods in different water temperature by using disc diffusion technique. The average resistance rates of the isolates to the antibiotics (CRO, ZOX & CTX) in the June- October 2007 were higher than that of the October, 2007-February, 2008. The highest resistance was detected to the ZOX and the lowest one was to the CRO in both periods. *K. pneumonia* showed the highest resistance to all three antibiotics compared to the *E. aerogenes* and *E. coli*. After plasmid curing, *K. pneumonia* has lost resistance at the highest level (52.72% to CRO, 44.72% to ZOX, 68.72% to CTX) compared to *E. coli* and *E. aerogenes*.

Key Words: Antibiotic; Resistance; Plasmid; Enterobacteriaceae

INTRODUCTION

Water constitutes a way of dissemination of antibiotic-resistant organisms among human and animal populations, the route by which resistance genes are introduced in natural bacterial ecosystems. In such systems, non-pathogenic bacteria could serve as a reservoir of resistance genes and platforms (Baquero *et al.*, 2008).

The presence of antibiotic resistant bacteria in water sources throughout the world has been documented (Kelch & Lee, 1978; French *et al.*, 1987; Ogan & Nwiika, 1993; Young, 1993). The majority of the studies focused on transferable drug resistance because of its practical importance (Pitout *et al.*, 1998; Stapleton *et al.*, 1999; Byarugaba, 2004). Resistance factors have been found in different host environments such as intensive care units (Pitout *et al.*, 1998), lagoon (Kish & Lampky, 1983), intestine (Salyers *et al.*, 2004) and in a variety of different organisms but mainly in the *Enterobacteriaceae* members. Water and sewage are the ones, which have been identified as reservoirs of enteric bacteria for spread of resistance factors (R-factors) (Bell *et al.*, 1983; Finch & Smith, 1986; Alonso *et al.*, 1996). The widespread use of antibiotics has helped to foster a remarkable type of resistance in bacteria (Shouten *et al.*, 1999). Emergence of resistance to third generation cephalosporins has been reported on strains of *Klebsiella* (Shah & Stille, 1983; Labia *et al.*, 1986) and transferable resistance to cefotaxime was demonstrated

already (Sirot *et al.*, 1987; Spencer *et al.*, 1987).

Resistance to β -lactam antibiotics among the *Enterobacteriaceae* members is the most commonly expressed by the production of β lactamases (Sanders & Sanders, 1986; Pitout *et al.*, 1998). The mechanism of resistance involved is the transferable plasmid mediated β lactamases has been described, in multi-resistant *K. pneumonia* (Sirot *et al.*, 1987). Natural reservoirs of resistance genes may also provide a source of transferable traits for emerging pathogens (Davies, 1994; Van Elsas *et al.*, 2000). It is also found that microorganisms resistant to antibiotics in Turkey's water sources and sewages. Although the prevalence, nature, properties of such reservoirs in Turkey has not been studied on a national scale, these regional studies will form interpretative and supplementary data for future works.

Here in this study, antibiotic resistance of some *Enterobacteriaceae* members, *Klebsiella pneumonia*, *Escherichia coli*, *Enterobacter aerogenes*, in hospital sewage was monitored and the variation of resistance to cephalosporin group antibiotics (ceftriaxone, cefotaxime & ceftizoxime) in different seasons was investigated.

MATERIALS AND METHODS

Isolation of bacterial strains. Water samples were collected on five sampling weeks between October 2006-February 2007 (First Period) and June- October 2007

(Second Period) from sewage of Cukurova University Hospital. Temperature and pH measurements were also recorded at the sampling time. All samples were transported in a refrigerated state to the laboratory.

The microorganisms were isolated every day for five weeks intervals in both periods. All samples, either diluted or undiluted in Luria-Bertani (LB) Broth, were plated on Endo-C and McConkey agar. After incubation at 37°C, the bacterial colonies appeared were identified by their colony morphology and biochemical analysis (Holt *et al.*, 1994).

Selection of resistant strains and elimination of plasmids.

For antibiotic resistance determination, the isolates were grown in LB until the turbidity equal to the 0.5 Mc Farland standard. Cultures were swabbed on to the Mueller-Hinton agar and all isolates were tested against Ceftriaxone (CRO)- (Fako, Actavis), Cefprozime (ZOX)- (Eczacibasi), Cefotaxime (CTX)- (Oxoid) antibiotic discs at final concentration of 30 µg mL⁻¹. The strains those grown in inoculation were evaluated as resistant and the others were evaluated as susceptible (Wang *et al.*, 1998; Malika *et al.*, 2004; Rushdy *et al.*, 2007). Experiments were also conducted to determine if antibiotic resistance was encoded on plasmid or not by plasmid elimination method. Ethidium bromide (100 µg mL⁻¹) was used for elimination of the plasmids from the strains (Poppe & Gyless, 1988).

RESULTS

The distribution of isolated strains (*E. coli*, *K. pneumoniae* & *E. aerogenes*) by weeks in both period and the percentage of antibiotic resistance are shown in Table I and Table II.

Total 655 bacterial samples were isolated on October, 2006- February, 2007. *E. coli* was not the predominant strain (38.04%), compared to the *E. aerogenes* (33.69%) and *K. pneumoniae* (27.02%) in the first week of the October 2006-February 2007 period. Similar results were obtained in the second and the fifth weeks of study. But in the third week, *E. coli* was the more predominant strain (69.14%) than *E. aerogenes* (15.95%) and *K. pneumoniae* (14.89%), while the lowest percentage of *E. coli* (9.77%) was obtained in the fourth week. Overall percentage of the strains isolated in first period was 35.87% (*E. coli*), 37.09% (*E. aerogenes*) and 27.02% (*K. pneumoniae*) (Table I). The water temperature was recorded as 12°C in first period and 32°C in the second period of the study.

A Total of 802 *E. coli*, *E. aerogenes* and *K. pneumoniae* were isolated in the June-October period. Total number and the percentage of the strains are also shown in Table II. There were no significant difference in the percentage of strains monitored weekly but overall percentage of the strains in five study weeks were fairly similar to the October 2006- Feb 2007 period (Table II). If both periods considered the distribution of the tested strains (1457) were 35.35%, 33.63%, 31% for *E. aerogenes*, *K. pneumoniae* and *E. coli*, respectively. It was found that the rates of the antibiotic resistance in the all strains vary in

Table I. Distribution of Isolates and Resistance to Antibiotics by weeks in October 2006 to February 2007

Isolates	Water Temp: 12°C		% of Strains by Weeks	(% Resistance to		
	Weeks	TNI		CRO	ZOX	CTX
<i>E. coli</i> (35.87%)	1	70	38.0	50	59	46
	2	57	39.0	11	12	7
	3	65	69.1	14	46	31
	4	13	9.8	s	s	s
	5	30	30.6	3	3	s
	Total	235	Av.Res	15.6	24	16.8
<i>E. aerogenes</i> (37.09%)	1	62	33.7	15	23	10
	2	58	39.7	2	7	2
	3	15	16.0	s	s	s
	4	64	42.1	19	19	16
	5	44	44.9	57	61	61
	Total	243	Av.Res	19.4	22	17.8
<i>K. pneumoniae</i> (27.02%)	1	52	28.3	71	75	67
	2	31	21.2	23	42	23
	3	14	14.9	14	7	14
	4	56	42.1	4	2	2
	5	24	24.5	25	46	8
	Total	177	Av.Res	27.4	34.4	22.8

TNI:Total Number of Isolates; s:Sensitive; CRO: Ceftriaxone; ZOX:Cefprozime; CTX: Cefotaxime; Av.Res:Average of total five weeks resistance rates

Table II. Distribution of Isolates and Resistance to Antibiotics by weeks in June-October 2007

Isolates	Water Temp:32°C		% of Strains by Weeks	(% Resistance to		
	Weeks	TNI		CRO	ZOX	CTX
<i>E. coli</i> (27.05%)	1	27	39.7	81	89	63
	2	40	21.9	38	38	33
	3	34	18.6	26	35	29
	4	56	39.4	21	25	25
	5	60	26.5	17	17	17
	Total	217	Av.Res	36.6	40.8	33.4
<i>E. aerogenes</i> (33.91%)	1	10	14.7	60	100	100
	2	62	33.9	27	27	26
	3	69	37.7	22	28	26
	4	50	35.2	14	20	12
	5	81	35.8	17	36	17
	Total	272	Av.Res	28	42.2	36.2
<i>K. pneumoniae</i> (39.2%)	1	31	45.6	26	55	55
	2	81	44.3	17	15	17
	3	80	43.7	58	60	49
	4	36	25.4	69	81	75
	5	85	37.7	24	24	24
	Total	313	Av.Res	38.8	47	44

TNI:Total Number of Isolates; s:Sensitive; CRO: Ceftriaxone; ZOX:Cefprozime; CTX: Cefotaxime; Av.Res:Average of total five weeks resistance rates

Table II. Resistance Loss after Plasmid Elimination (%)

	CRO		ZOX		CTX	
	Oct-Feb	Jun-Oct	Oct-Feb	Jun-Oct	Oct-Feb	Jun-Oct
<i>E. coli</i>	41.09	36.81	28.36	5.90	43.63	33.17
<i>E. aerogenes</i>	28.36	26.81	14.18	14.09	32.72	22.72
<i>K. pneumoniae</i>	52.72	14.54	44.72	10.00	68.72	16.36

periods. In the first period, almost half of the *E. coli* strains had resistance to Ceftriaxone (CRO), Cefprozime (ZOX) and Cefotaxime (CTX), but the resistance were rare in the fifth week ($\leq 3\%$). The highest level of resistance to these

antibiotics was detected to be more than 65% in the *K. pneumonia* in the first week of the period. But, *E. aerogenes* was developed resistance against to these antibiotics ($\geq 57\%$) compared with the resistance rates of the first week.

In the first period, the highest level of resistance to CRO, ZOX and CTX were detected to be 27.4%, 34.4% and 22.8% of the *K. pneumonia*, respectively (Table I). On the other hand, all strains were found to be more resistant to ZOX antibiotic than CRO and CTX.

In the second period of study, *E. coli* and *E. aerogenes* showed a gradual resistance decrease to CRO, ZOX, CTX, but *K. pneumonia* presented an increase in acquired resistance to these three antibiotics for the fourth week of the study. If the appeared resistance rates of strains compared by week, *E. aerogenes* was the one showing the highest resistance to ZOX (100%) and CTX (100%) in the first week of the second period. In terms of resistance to CRO antibiotic, *E. coli* was the most resistant (81%) compare to others.

As shown in Table I and II, the mean resistance rates of the June- October 2007 are higher than that of the October, 2006-February, 2007. When five weeks total resistance rates considered *K. pneumonia* showed the highest resistance to all three antibiotics (CRO: 38.8%, ZOX: 47%, CTX: 44%) compared to the *E. aerogenes* and *E. coli*. The loss of resistance in *E. coli* and *E. aerogenes* in the period of June- October 2007 were found to be 36.81% and 26.81% to CRO, 5.90% and 14.09% to ZOX and 33.17% and 22.72% to CTX, respectively. On the other hand, *K. pneumonia* lost its resistance with lower ratios (14.54% to CRO, 10.00% to ZOX, 16.36% to CTX) compared to those bacteria isolated in the period of October, 2006- February, 2007, un-expectedly (Table III).

DISCUSSION

Bacterial resistance to antimicrobial agents are increasing worldwide due to several reasons (Sanders & Sanders, 1997). One of them has been linked to the over-use of antibiotics especially in intensive care units (Jarlier *et al.*, 1988). The resistant strains were not only found in the hospital environment but also have been spread to the environment such as receiving water, treatment plants, agricultural areas via sewage water. So the genetic exchange between bacteria in the environment cannot be undervalued for emergence of new resistant strains. In water, bacteria from different origins (human, animal, environmental) are able to mix and resistance evolves as a consequence of promiscuous exchange and shuffling of genes, genetic platforms, and genetic vectors (Baquero *et al.*, 2008).

That the presence of antibiotic resistant bacteria in sewage (Altherr & Kasweck, 1982; Kümmerer, 2001) and surface water (Wnorowski, 1993) is a growing public health concern had already been stressed. In the present study, we assessed the antibiotic resistance rates among some *Enterobacteriaceae* members in sewage water at two

different seasons. The average water temperature was 12°C during the October-February period but 32°C in June-October and the pH of the water were 6.5 and 7.4, respectively. The numbers of the bacteria recovered were totally 1457 in both periods. The results shows that there is a significant increase in the antibiotic resistance rates of all strains tested in June-October, 2007 period compared to strains isolated in the October, 2006- February, 2007. This indicates that the water temperature is an essential factor for resistance development (Jarlier *et al.*, 1988). On the other hand, lower water temperature could be a restricting factor for the transfer of resistance gene in the winter. Then *et al.* (1983) and Jarlier *et al.* (1988) indicated that conjugation takes place at a lower level with a low temperature below 15°C.

The resistance to CRO, ZOX and CTX in this study strongly suggest that *E. coli*, *K. pneumonia*, *E. aerogenes*, are the strains that producing extended-spectrum β lactamase (ESBL). *K. pneumonia* was known as the first ESBL producing strain until the early 1990's (Katsanis *et al.*, 1994; Livermore, 1995; De Moüy *et al.*, 1997; Nordman, 1998; Jarlier & Nordmann, 2000; Livermore *et al.*, 2001, Gniadkowski, 2001). So, it is not surprising that *K. pneumonia* show the highest rate of resistance to all tested cephalosporins than the other strains. The increase in resistance could be explained with conjugation taking place between the bacteria. These results suggest that infection control protocol should deal antibiotic susceptibility test before the therapy is started.

If a strain is still resistant with a remaining rate in spite of a plasmid elimination procedure (Table III), the only interpretation about the result is that the resistance genes could be found on mega plasmids or incorporated into the bacterial chromosome. It is often the case that plasmids have played a role in bringing the new genes into the host cell and an incoming plasmid can be stably incorporated into the chromosome or the relevant genes contained in a transposon can jump onto the chromosome before the plasmid is lost (Rajakumar *et al.*, 1997; Briggs & Fratamico, 1999; Hughes & Anderson, 2001).

The existence of these resistance bacteria in sewage water, no matter where they come from, is a real risk for acquiring such bacteria in the environment. Since sewage water is an important reservoir for possibility of resistant transfer of genes, in which bacteria concentration is high and thus the change of contact between two suitable bacteria is high (Feuerpfeil, 1999). Contribution and precautions should be increased at least for dissemination of these resistant bacteria in the environment.

In conclusion, the results of this study underline the importance of the water temperature for dissemination of the resistance factors between the microorganism and such a screening of antibiotic resistance may be reflecting the consequence of the drug using habits and would help to address the contribution spread of resistant bacteria to the environment.

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