Antibiotic resistant *E.coli* in drinking water: resistance to quinolones and 2nd generation cephalosporins

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Abstract

We investigated the antibiotic resistance of 133 *E.coli* strains from water used for drinking purposes in towns and small rural communities on three islands in Greece. We measured the resistance to tree quinolones (Norfloxacin 10μg- NXN, Ciprofloxacin 5μg - CIP, Levofloxacin 5μg - LVX) and two cephalosporins (Amoxicillin 25μg - AMX, Cefaclor 30μg - CEC). The percentage of strains resistant to the cephalosporins tested was: 29.3% resistant to AMX, 10.5% to CEC. Resistance to quinolones was smaller: 11.3% to LVX, 0.8% to CIP and none resistant to NXN. Multiple resistance was observed: 5.2% to three antibiotics, 9.0% to two antibiotics. All resistant strains came from small rural water sources. It appears that there is a significant reservoir of cephalosporin resistant *E.coli* in water used for drinking, and that resistance is building for quinolones.

Introduction

Antibiotic resistant bacteria are universally considered a public health threat, as they reduce the efficacy of antibiotics. They place substantial clinical and economic burden to societies, and the loss of lives is not beyond consideration. Antibiotic resistant bacteria have been long isolated from environmental sources and agricultural practices, with consequences on the food chain and human health, as the resistance can disseminate to humans. The existence of resistant feacal bacteria in aquatic environment has been documented (Garcia-Armisen 2011). Antibiotic resistant *E.coli* from surface and ground water, entering the potable water distribution system, if poorly disinfected, can pose a threat to public health. The prevalence of sulphonamides, tetracycline, chloramphenicol and penicillins resistant genes in drinking water has been described (Guo et al 2014, Khan et al 2016, Xu et al 2016). Some of the most powerful antibiotics used today are those belonging to the groups of quinolones and 2nd generation cephalosporins. Little has been published on cefalosporin and/or quinoline resistant bacteria from drinking water sources (Jiang et al 2013). Coliform resistance to amoxicillin from river water was investigated, and resistance to this antibiotic was observed in 20,9% of the examined strains (Stange et al 2016).
In this research project we sought to investigate the presence of resistance to five antibiotics (2nd generation cephalosporins and quinolines) of *E.coli* strains isolated from drinking water sampled in three greek islands. The water came brom urban distribution systems and from rural communities (wells and spring water).

Materials & Methods

1.Samples were taken in sterile glass bottles and transported refrigerated to the Laboratory within 24h of collection.
2.Filtration (V=100mL) through a membrane filter (diameter 47mm), pore size 0,45μm.
3.Membranes cultured on Chromogenic Coliform Agar. Incubation at 36±2°C for 21±3h.
4.β-D-glucuronidase positive colonies (dark-blue to violet color) transferred on Tryptic Soy Agar. Incubation at 36±2°C for 21±3h.
5.Oxidase negative colonies transferred to Tryptone Water to perform Indole test. Incubation at 44±0,5°C for 21±3h.
6.Colonies positive for Indole test and for β-D-glucuronidase were considered as *Escherichia coli*, and were spread on Mueller Hinton Agar (Kirby – Bauer disk diffusion susceptibility test). Antibiotic disks Bio-Rad. Incubation at 35±1°C for 16±4h.
For reference purposes strains of *Escherichia coli* NCTC 9001 were used (Lenticulers Sigma-Aldrich).

Results and Discussion

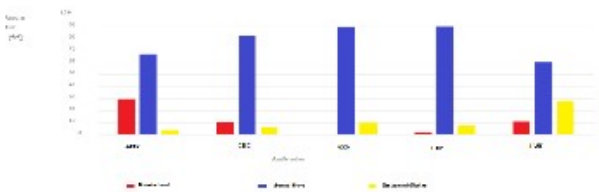
Of the 133 *E.coli* isolates examined, 45 (33.8%) were resistant to at least one of the antibiotics. Amoxicillin was the antibiotic to which the largest percentage (29.3%) of *E.coli* were resistant, followed by Levofloxacin (11.3%) and Cefaclor (10.5%). To Ciprofloxacin the resistant strains were 4.5%, and zero resistance was observed for Norfloxacin. Intermediate resistance was at 3.8 – 4.5% for four of the examined antibiotics. An interesting finding is that 27.8% of the strains showed intermediate resistance to Levofloxacin, a comparatively new antibiotic, second-generation fluoroquinolone, which was approved for medical use in Japan in 1993 and the USA in 1996. 4.5% of the isolates presented resistance to three antibiotics, 9.8% to two antibiotics. The two isolates that were CIP resistant were resistant to LVX, too. The strains resistant to three antibiotics were always resistant to the two cephalosporins and Levofloxacin.
All resistant strains of *E.coli* were isolated from water sources (wells and springs) of small rural communities. The same for strains showing intermediate resistance. The sensitive strains were isolated from treated drinking water from urban distribution systems. This agrees with the results of Jiang et al (2013) who observed antibiotic resistance to be more severe in strains isolated from rural areas.
This study demonstrated that excessive use of antibiotics in animal feed as well as increased use in treating humans has led to a contamination of the aquatic environment with antibiotic resistant strains of microorganisms. Drinking water isolates of *E.coli* can be a reservoir of antibiotic resistance genes in widely used antibiotics, and to the best of our knowledge it is the first documented isolation of drinking water *E.coli* strains resistant to ciprofloxacin and levofloxacin. Evidence is provided for the need to limit the spread of antibiotic resistant bacteria in water, and for the development of management strategies to that effect.

Antibiotic zone of inhibition (diameter)

Antibiotic concentration	Sensitive (cm)	Intermediate (cm)	Resistant (cm)
Amoxicillin (AMX25μg)	≥1,7	1,4-1,6	≤1,3
Cefaclor (CEC30μg)	≥1,8	1,5-1,7	≤1,4
Norfloxacin (NXN10μg)	2,5-3,1	2,1-2,4	≤2,0
Ciprofloxacin (CIP5μg)	2,8-3,5	2,5-2,7	≤2,4
Levofloxacin (LVX5μg)	2,9-3,6	2,6-2,8	≤2,5

Numbers (percentage) of antibiotic sensitivity of *E.coli* isolates

Number of strains examined 133	AMX (Amoxicilin)	CEC (Cefaclor)	NXN (Norfloxacin)	CIP (Ciprofloxacin)	LVX (Levofloxacin)
Sensitive	89 (66,9%)	110 (82,7%)	127 (95,5%)	124 (94,7%)	81 (60,9%)
Intermediate	5 (3,8%)	9 (6,8%)	6 (4,5%)	7 (4,5%)	37 (27,8%)
Resistant	39 (29,3%)	14 (10,5%)	0 (0%)	2 (0,8%)	15 (11,3%)



Antibiotic multiple resistant isolates

Multiple resistant strains of E.coli	Isolates resistant to AMX	CEC	NXN	CIP	LVX
6	✓	✓			✓
4	✓				✓
7	✓	✓			
2				✓	✓
Total: 19					

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