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Plasmid profiling and antibiotics resistance of *Escherichia coli* strains isolated from *Mytilus galloprovincialis* and seawater

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ABSTRACT

Objective: To investigate plasmid DNA profiles and the antibiotic resistance of a total of 41 strains of *Escherichia coli* (*E. coli*) isolated from seawater and mussel collected from 15 different sampling stations in Sinop, Turkey.

Methods: Most probable number technique was used for detection of *E. coli*. Antibiotic susceptibilities of the isolates were determined by the disc diffusion method. Plasmid DNA of the strains was extracted by the alkaline lyses procedure.

Results: According to morphological and physiological properties, it was determined that the isolates belonged to *E. coli* species. Antibiotic susceptibility of the strains was determined against seven standard drugs using disc diffusion method. All isolates were resistant to bacitracin (100%), novobiocin (100%), ampicillin (12.5%), tetracycline (7.5%), ceftazidime (5%) and imipenem (2.5%), respectively, whereas the strains were susceptible to polymyxin B (100%). The multiple antibiotic resistance values for the strains were found in range from 0.28 to 0.57. In addition, plasmid DNA analyses results confirmed that 22 strains harbored a single or more than two plasmids sized approximately between 24.500 to 1.618 bp. The high-size plasmid (14.700 bp) was observed as common in 21 of all strains.

Conclusions: As a result, our study indicated that the presence of antibiotic resistant *E. coli* strains in seawater and mussel might be potential risk for public health issue.

1. Introduction

Escherichia coli (*E. coli*) is the most important fecal indicator microorganism in seafood and marine aquatic environments. The presence of this bacterium in saline habitats is a possible predictor to existence of other enteric pathogens[1-3]. *E. coli* is a member of the normal microflora in the gastrointestinal tract of human and warm blood animals[4,5]. Nowadays, the prevalence of fecal contamination in water reservoirs is a very important problem in developing and developed countries[6]. Water-borne and seafood-borne pathogenic *E. coli* strains cause serious diseases threatening human health, such as diarrhea, hemorrhagic colitis, hemolytic-uremic

syndrome, severe abdominal pain, which may even be fatal[7,8].

Multidrug resistance bacterial pathogens can be accumulated in several ecological niches because of the widespread use of commercial antibiotics in hospital, agriculture and livestock. Multiple antibiotic resistances (MARs) in bacteria may be commonly associated with the presence of plasmids[9]. Plasmids are circular double-stranded extra-chromosomal DNA molecules, and conjugal transfers of plasmids play an important role in the spread of antibiotics resistant genes among *E. coli* and other bacterial strains. The resistance to commonly using antibiotics in bacteria creates a threat to public health in the world. In addition, the size, number and attributes of the plasmid in a bacterium remain the same for a long time and they are transferred to equally daughter cells. Therefore, plasmids have an important role in epidemiological and taxonomic studies[10].

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Sinop is our main research area where intensely used for recreation activities, swimming, fishing and mussels harvesting during the all seasons. The city sewage is often discharged directly or indirectly into the sea from various sources along the coast. The aim of the study was to examine the plasmid DNA profiling and antibiotic resistance patterns of *E. coli* isolated from mussel and seawater samples collecting Sinop environs (Black Sea region, Turkey) between May 2011 and October 2011.

2. Materials and methods

2.1. Samples

Seawater samples were taken from 10 sampling sites in coastal areas of Sinop from May 2011 to October 2011. Mussel samples were also collected from 5 sampling sites between the same dates. Seawater samples were taken 10² mL sterile bottles directly from the undersurface. Mussels were harvested by hand and scoop in coastal reefs. All samples protecting to approximately 4 °C freezers were brought to the laboratory within 3 h. Mussel samples were washed in sterile distilled water to remove epiphytes on the shell. The collected samples were prepared for planting under aseptic conditions. Most probable number technique was used for detection of *E. coli*[11].

2.2. Characterization of *Escherichia coli* isolates

Following most probable number, 100 µL samples taken from the positive tubes were inoculated to *E. coli* broth (Merck) and were incubated for 24 h at 44.5 °C. Then, gas positive tubes were selected as presumptive isolates of *E. coli*. The isolates were grown again on eosine methylene blue (Merck) agar plates to observe for green metallic sheen production as confirmatory test. The characterizations of a total of 40 native isolates of *E. coli* were carried out according to Welch[12] and Brenner and Farmer[13].

2.3. Antimicrobial susceptibility testing

Antibiotic susceptibilities of the isolates were determined by the disc diffusion method[14] using Luria Bertani agar (Oxoid) and seven antibiotic discs: bacitracin (0.04 IU), ceftazidime (30 µg), imipenem (10 µg), novobiocin (5 µg), polymyxin B (300 IU), tetracycline (30 µg) and ampicillin (10 µg). *E. coli* ATCC 25922 was used as control organism, and the results were explained using Clinical and Laboratory Standards Institute criteria[15]. We calculated the MAR index values for all isolates[16].

2.4. Plasmid isolation and screening

Plasmid DNA of the strains was extracted by the alkaline lyses procedure[17]. Some steps in procedure were performed in ice. All bacterial strains were incubated overnight in 5 mL Luria Bertani broth at 37 °C in a hot water bath shaker. The bacterial cells were collected by centrifugation at 10 000 r/min for 1 min in a 1.5 mL micro centrifuge. The supernatant was carefully discarded and the pellet thoroughly resuspended and vortexed in 100 µL of glucose–Tris–EDTA buffer. Then, 200 µL of lysis buffer solution was added and the mixture was gently vortexed and incubated for 15 min on ice. Subsequently, centrifuged at 10 000 r/min for 7 min and supernatant was transferred to a sterile eppendorf tube. Equal volume of phenol: chloroform was added and vortexed. Centrifuged at 10 000 r/min for 5 min and supernatant was transferred to a clean Eppendorf tube. The extracted plasmid DNA was precipitated and concentrated by using 96% and 70% alcohol respectively. Then, alcohol was removed and dissolved in 50 µL of Tris–EDTA buffer. A 0.7% agarose was prepared in 1× Tris–borate–EDTA and mixed with 0.5 µg/mL of ethidium bromide. The gel was transferred to electrophoresis unit. Each sample was mixed with loading dye and loaded into the gel and 1 µL DNA marker (Fermantas) was loaded into one well. Then, samples were electrophoresed for 2.5–3 h at 80 V in 1× Tris–borate–EDTA buffer and the gel was photographed under UV transilluminator using Cleaver–Microdoc, UK.

3. Results

According to morphological and physiological properties, it was determined that the local isolates belonged to *E. coli* species. In the study, a total of 41 *E. coli* strains identifying as *E. coli*, 40 native isolates and 1 reference strain were evaluated for antibiotic susceptibility by using the disc diffusion procedure. The results of antibiogram showed that the strains were the resistance to at least two or more antibiotics (Table 1).

Table 1
Antibiotic resistances (%) of *E. coli* strains.

Antibiotics	Resistant	Intermediate	Sensitive
Bacitracin	100.0	–	–
Novobiocin	100.0	–	–
Tetracyclin	7.5	–	92.5
Ampicillin	12.5	35.0	52.5
Imipenem	2.5	22.5	75.0
Ceftazidime	5.0	30.0	65.0
Polymyxin B	–	–	100.0

The antimicrobial resistance patterns of *E. coli* strains are shown in Tables 1 and 2. All of the isolates were found resistance to bacitracin and novobiocin, except for polymyxin B. The resistance to tetracycline, ampicillin, imipenem and ceftazidime was determined in 7.5%, 12.5%, 2.5% and 5%, respectively. Especially, one strain isolated from mussel was resistant to ampicillin, besides bacitracin and novobiocin. Our findings indicated that the multidrug resistance was higher among water-borne isolates than mussel isolates (Table 2).

Whole isolates were screened for the presence of the plasmid DNA, and it was found that 22 strains harbored a single or more than two plasmids sized approximately between 24.500 and 1.618 bp as summarized in Table 2 and Figure 1. The high-size plasmid (molecular weight 14.700 bp) was observed as common in 21 of all strains. In addition, plasmid DNA analysis revealed that there was no specific plasmid profiling among tested strains. Our findings also indicated that some isolates had multidrug resistance, although they were not including any plasmid.

Table 2
Resistance patterns and plasmid profiles of *E. coli* strains isolated from seawater and mussel.

Origin	Strain No.	Plasmid sizes (bp)	Plasmid No.	Drug resistances
W	1	14.700	1	Bc, Nov
W	2	24.500, 14.700	2	Bc, Nov, Amp, Cef
W	3	14.700	1	Bc, Nov
W	4	14.700	1	Bc, Nov
W	5	14.700	1	Bc, Nov
W	6	14.700	1	Bc, Nov
W	7	–	–	Bc, Nov
W	8	–	–	Bc, Nov
W	9	14.700	1	Bc, Nov
W	10	–	–	Bc, Nov

Table 2 continued

Origin	Strain No.	Plasmid sizes (bp)	Plasmid No.	Drug resistances
W	11	–	–	Bc, Nov
W	12	6.545, 3.792, 3.116	3	Bc, Nov
W	13	–	–	Bc, Nov
W	14	–	–	Bc, Nov
W	15	–	–	Bc, Nov
W	16	–	–	Bc, Nov
W	17	–	–	Bc, Nov, Amp, Cef
W	18	–	–	Bc, Nov
W	19	14.700	1	Bc, Nov, Amp
W	20	14.700	1	Bc, Nov
W	21	14.700, 5.650	2	Bc, Nov
W	22	14.700, 5.650, 4.631	3	Bc, Nov
W	23	14.700	1	Bc, Nov
W	24	–	–	Bc, Nov
W	25	14.700	1	Bc, Nov
W	26	–	–	Bc, Nov
W	27	14.700	1	Bc, Nov
W	28	12 plasmids (14.700–1.618)	12	Bc, Nov, Te, Amp
W	29	–	–	Bc, Nov
W	30	–	–	Bc, Nov
W	31	14.700, 5.523, 4.180	3	Bc, Nov
W	32	–	–	Bc, Nov, Te, Amp
W	33	14.700	1	Bc, Nov
W	34	14.700	1	Bc, Nov, Te
M	35	–	–	Bc, Nov, Amp
M	36	–	–	Bc, Nov
M	37	20.973, 17.081, 14.700	3	Bc, Nov
M	38	–	–	Bc, Nov
M	39	–	–	Bc, Nov
M	40	11.895	1	Bc, Nov
Control	K	14.700	1	Bc, Nov, Imp

W: water, M: mussel, K: *E. coli* ATCC 25922, Bc: Bacitracin, Nov: novobiocin, Amp: ampicillin, Te: tetracycline, Cef: ceftazidime, Imp: imipenem.

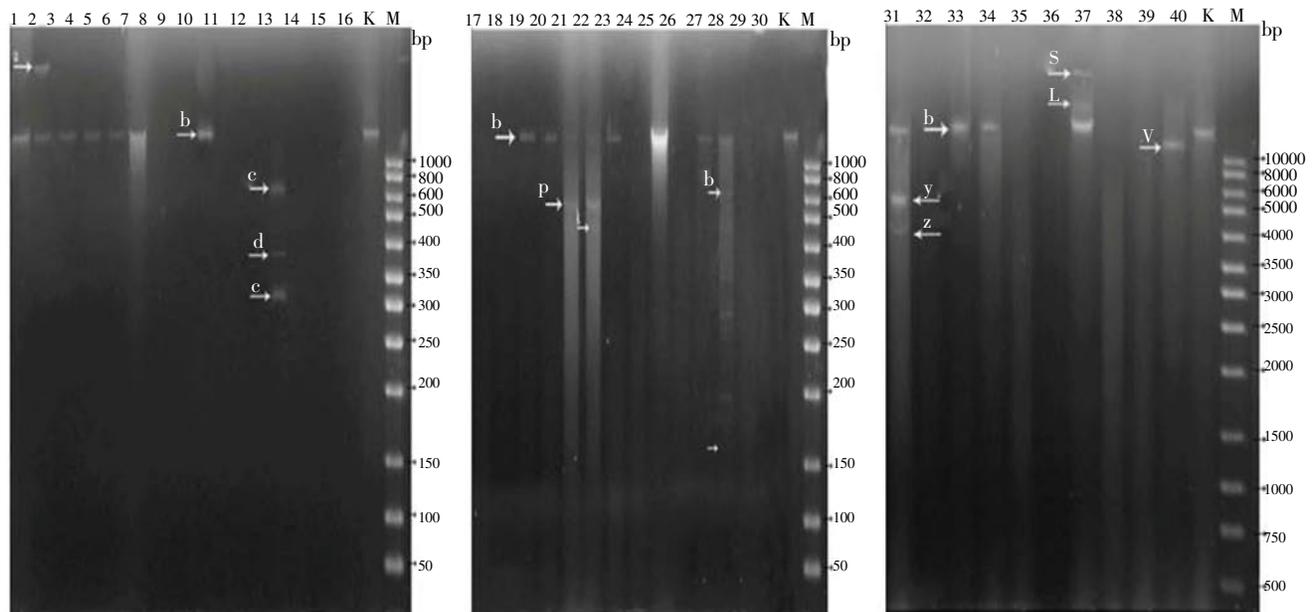


Figure 1. Plasmid profiles of *E. coli* strains isolated from seawater and mussels. K: *E. coli* ATCC 25922; M: Marker (10000 bp– Fermantes).

4. Discussion

A number of the pathogenic microorganisms contaminate to coastal waters and other aquatic habitats as a result of human and animal activities[18]. The pathogens in water and seafood cause serious diseases threatening human health, such as diarrhea, dysentery, typhoid, cholera and hemolytic–uremic syndrome. In the study, we investigated the rate of antimicrobial resistances and plasmid profiles of *E. coli* strains isolated from seawater and mussel samples harvesting Sinop environs intensively used for recreational and other human activities.

In our study, the physiological and biochemical tests were successfully applied to identify *E. coli* strains isolated from seawater and mussels. The morphological and physiological properties of the isolates confirmed that they belonged to *E. coli* species. According to the results of antibiogram, all tested isolates in the study were resistant to bacitracin and novobiocin; whereas they were susceptible to polymyxin B. In addition, the resistance to tetracycline, ampicillin, imipenem and ceftazidime was determined in 7.5%, 12.5%, 2.5% and 5%, respectively. Our results are agreed with the findings of Sharma and Rai who determined to resistance against ampicillin (55%) and ceftazidime (45%) in 11 *E. coli* strains isolated from lake water[19].

Shar *et al.* reported that the *E. coli* strains isolated from drinking water were resistant to bacitracin and susceptible to imipenem[20]. Patoli *et al.* found that the 27 *E. coli* strains were resistant to ampicillin (88.89%), ceftazidime (25.23%) and shown to MAR (3–6 antibiotics) of 62.96% of the strains[21]. Lösch *et al.* determined that the 68 *E. coli* strains isolated from water samples were resistant to ampicillin (44.1%)[22]. Danishta *et al.* stated that the 19 *E. coli* strains isolated from wastewater and environ samples were resistant to tetracycline (31.6%) and ceftazidime (10.5%)[23]. Vignaroli *et al.* found that the 109 *E. coli* isolates isolated from seawater coast sediment were resistant to tetracycline (28%) and ampicillin (16.5%)[24]. Akter *et al.* determined that the 163 *E. coli* isolates were resistant to ampicillin (86.5%), tetracycline (77.30%) and imipenem (5.52%)[25]. Aly *et al.* found that the highest sensitivity of the *E. coli* isolates was to polymyxin B and imipenem[26]. Our findings were in line with the results of the studies as mentioned at above.

On the other hand, Islam *et al.* determined that the 10 *E. coli* strains isolated from drinking bottle and tap water were resistant to tetracycline (90%), bacitracin (100%) and novobiocin (60%)[27]. According to Poongothai *et al.*[28], the 153 *E. coli* strains were resistant to novobiocin (92.1%), ampicillin (87.5%), tetracycline (81%) and bacitracin (70.5%). These results are not compatible with our results. MAR

indexes for all strains were also found in range from 0.28 to 0.57. The calculated MAR values showed that seven of screened isolates had multiple resistances to at least three or four antibiotics.

In the study, the high varieties of plasmid profiles were extracted from the isolates. The results of the plasmid DNA analysis displayed that the 21 strains contained plasmids, and the other 19 strains did not include any plasmids. The plasmid–free 19 isolates were found resistance to several antibiotics, such as bacitracin, novobiocin, ampicillin, tetracycline and ceftazidime. The distribution of plasmids in all strains appeared that there was no specific plasmid profiling among examined isolates. This result confirmed that there was no a linear correlation between the plasmids content and the antibiogram studies of the isolates. In this vain, our findings were coherent with as mentioned previous studies[29–31].

The results of this study suggested that the coastal waters of Sinop environs used for recreation activities and mussels harvesting contained at high levels multiple antibiotic resistant *E. coli* isolates during the summer time seasons. Our results proposed that there was not a correlation between the presence plasmid and multidrug resistance due to the presence of free–plasmid isolates having antibiotic resistance. This case almost indicted that multidrug resistant determinants were located in chromosomal DNA instead of plasmid. As a result, we underline the need for monitoring the presence of multidrug resistant bacteria to protect from water–borne and seafood–borne infections.

Conflict of interest statement

We declare that we have no conflict of interest.

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