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RESEARCH ARTICLE

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Escherichia coli isolated from seafood: toxicity and plasmid profiles

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Abstract Thirty-two *Escherichia coli* strains were isolated from red snapper (*Lutjanus purpureus*) and from seabob shrimp (*Xiphopenaeus kroyeri*). The strains were numbered S1–S16, and F1–F16, which corresponds to the isolation origin from shrimp (S) and fish (F). The isolates were biologically and antigenically characterized by agglutination tests with enteropathogenic *E. coli* (EPEC)-, enteroinvasive *E. coli* (EIEC)- and enterohemorrhagic *E. coli* (EHEC) O157-specific antisera. The ETEC enterotoxins were characterized by GMI-ELISA for enterotoxin LT-1 (thermolabile) and by inoculation of supernatants prepared from newly born mice for enterotoxin Sta. A total of 14 strains produced exotoxins, of which seven were thermolabile (LT) and seven were thermostable (ST). Antimicrobial susceptibility profiles were determined by disc diffusion in agar using ampicillin, cephalothin, cefoxitin, ceftriaxone, imipenem, nalidixic acid, ciprofloxacin, chloramphenicol, gentamicin, nitrofurantoin, sulfamethoxazole–trimethoprim, and tetracycline. Four isolates showed lower susceptibility to some antibiotics, two strains were resistant to ampicillin, tetracycline, and sulfamethoxazole-trimethoprim, and two were resistant to tetracycline and nitrofurantoin. Plasmids were extracted in the four resistant isolates; two of them contained plasmids whose molecular weight varied from low to high. The characterization of LT- and ST-toxin-producing *E. coli* strains displaying multiresistance and containing plasmids suggests the need for tightening current control measures for the use of antimicrobials.

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Introduction

Escherichia coli is a major component of the intestinal microbiota in both humans and other animals. Colonies develop a few hours after the animal's birth, but remain inoffensive as long as they are confined to the intestinal lumen. However, in a weak or immunosuppressed host, or in the case of injury to gastrointestinal barriers, even non-pathogenic strains may cause infection [12]. *E. coli* strains can cause a variety of diseases, including diarrhea, dysentery, hemolytic uremic syndrome, and bladder and kidney infections. Different strains are usually associated with different diseases; this versatility of *E. coli* strains is due to the fact that different strains have acquired different sets of virulence genes. At least five biotypes are currently known to induce intestinal infection: enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAggEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), and enteroinvasive *E. coli* (EIEC). ETEC strains resemble *Vibrio cholerae* in that they adhere to the small intestinal mucosa and produce symptoms not by invading the mucosa, but by elaborating toxins that act on mucosal cells to cause diarrhea. ETEC diarrhea can be fatal, especially in infants and young children, and is a major cause of death in this sector of the population in many developing countries [1, 11,16]. Adult ETEC diarrhea is commonly called traveler's diarrhea [3]. Humans are the reservoir for ETEC strains, which can be transmitted to other human beings through the consumption of water and food contaminated by feces. In this work, we evaluated the presence of different biotypes of *E. coli* in seafood, especially ETEC strains. The resistance profile of isolates to important clinical antibiotics, and whether this/these resistance(s) might be a consequence of, or be related to, the presence of plasmids were also examined.

Materials and methods

Samples

Ten specimens of red snapper (*Lutjanus purpureus*) and 3 kg of seabob shrimp (*Xiphopenaeus kroyeri*), purchased at the Mucuripe seafood market, in Fortaleza, Northeastern Brazil, on different days, were analyzed. Samples were frozen and sent to the laboratory in thermally isolated boxes.

Escherichia coli characterization

Agglutination assays following the US Food and Drug Administration (FDA) recommendations were carried out to characterize 32 *E. coli* isolates [8]. *E. coli* strains were submitted to antigenic analyses using slide agglutination tests with EPEC-, EIEC- and EHEC O157-specific polyvalent antisera, produced at the enterobacteriology laboratory of the Oswaldo Cruz Institute (FIOCRUZ, Rio de Janeiro, Brazil). The ETEC group was diagnosed by the enterotoxin assay, using GMI-ELISA immunoenzyme assay to detect enterotoxin LT-1 [7] and inoculation of supernatant prepared from cultures of newly born mice to detect enterotoxin Sta [6].

Antimicrobial testing

Susceptibility of the isolated strains to different drugs was determined by disc-diffusion agar as specified by the National Committee for Clinical Laboratory Standards [13]. The following drugs were tested: nalidixic acid (30 μg); ampicillin (10 μg); cephalothin (30 μg); cefoxitin (30 μg); ceftriaxone (30 μg); ciprofloxacin (5 μg); chloramphenicol (30 μg); gentamicin (10 μg); imipenem (10 μg); nitrofurantoin (300 μg); sulfamethoxazole–trimethoprim (1.25/23.75 μg); and tetracycline (30 μg).

Plasmid extraction

Plasmid DNA was extracted by alkaline lysis, as described by Birnboim and Doly [2] and modified by Sambrook et al. [15], and electrophoresed in a horizontal 0.7% agarose gel with pH 8.0 TBE buffer. The gels were subsequently stained with 0.5 mg/ml ethidium bromide for 20 min. Plasmid sizes were measured by comparison with migrating plasmids of known sizes (98, 42, 24 and 4.6 MDa) and with those present in the control strains *E. coli* 39R861 and *E. coli* V517 (35.8, 4.8, 3.7, 2.6, 2.0 and 1.8 MDa).

Results and discussion

Fish and shrimp samples collected at the seafood market in Mucuripe, Fortaleza, Brazil, yielded 32 *E. coli* strains. Sixteen of the strains were isolated from fish (F) and the other 16 strains from shrimp (S); they were numbered F1–16 and S1-S16, respectively. The isolation of potentially hazardous *E. coli* strains from seafood purchased at the market in Fortaleza was not an unexpected finding. In fact, Vieira et al. [22] had already detected enteropathogenic *E. coli* strains (EPEC) of serotypes O26, O127, O55 and O125 in samples of sea water collected off three beaches in Fortaleza; this area corresponds to the local shrimp fishing grounds, which are extensively exposed to raw sewage discharged into the ocean from the city. Other studies [20] have likewise isolated *E. coli* strains from seafood purchased at the same market, which indicates precarious sanitary conditions. The quality and/or storage condition of the ice used at the market may be an additional source of infection [21]. In addition, *E. coli* may seriously impair seafood quality because this bacterium can reduce trimethylamine oxide (TMAO) to trimethylamine (TMA), a nitrogenated base found in marine fish. TMA is frequently used as an indicator of muscle decomposition [5].

Neither EPEC nor EIEC serotype groups were found in the antigenic analyses; however, 14 isolates excreted enterotoxins (Table 1). ETEC strains may produce LT and/or ST enterotoxins. Thermolabile enterotoxin (LT) is very similar in structure and mode of action to cholera toxin produced by *Vibrio cholerae*, and infections with these strains can mimic cholera, particularly in young and malnourished children. Other ETEC strains produce thermostable enterotoxins (ST) in addition to LT. The enterotoxins detected in five *E. coli* strains from fish samples were of LT type. Whereas eight isolates from shrimp samples produced mainly the ST type, only one isolate was of LT type and one another produced both, LT and ST.

Four isolates showed lower susceptibility to some antibiotics. Strains S12 and S13 were resistant to ampicillin, tetracycline, and sulfamethoxazole-trimethoprim, whereas strains F15 and F16 strains were resistant to tetracycline and nitrofurantoin. Silva and Hofer [18] have described *E. coli* isolates from seafood that were resistant to antibiotics and heavy metals. However, in an analysis of a large number of *E. coli* strains isolated from seawater samples collected off three beaches in Fortaleza, Vieira et al. [22] did not find any strain resistant to either ampicillin, cephalothin, gentamicin, tetracycline, sulfamethoxazole-trimethoprim, chloramphenicol, or ciprofloxacin.

Plasmids were extracted from the four resistant-isolates. Strains F15 and F16 contained plasmids, but

Table 1. Detection of LT/ST in *Escherichia coli* isolated from seafood samples. *LT+* Detection of thermolabile toxin, *LT–* no detection; *ST+* detection of thermostable toxin, *ST–* no detection

Isolates from shrimp	Enterotoxins	Isolates from fish	Enterotoxins
S1	LT+ and ST–	F1	LT+ and ST–
S2	LT– and ST–	F2	LT– and ST–
S3	LT– and ST–	F3	LT– and ST–
S4	LT– and ST+	F4	LT– and ST–
S5	LT– and ST–	F5	LT– and ST–
S6	LT– and ST–	F6	LT– and ST–
S7	LT– and ST–	F7	LT+ and ST–
S8	LT– and ST–	F8	LT– and ST–
S9	LT+ and ST+	F9	LT+ and ST–
S10	LT– and ST+	F10	LT+ and ST–
S11	LT– and ST+	F11	LT– and ST–
S12	LT– and ST–	F12	LT+ and ST–
S13	LT– and ST+	F13	LT– and ST–
S14	LT– and ST+	F14	LT– and ST–
S15	LT– and ST–	F15	LT– and ST–
S16	LT– and ST+	F16	LT– and ST–

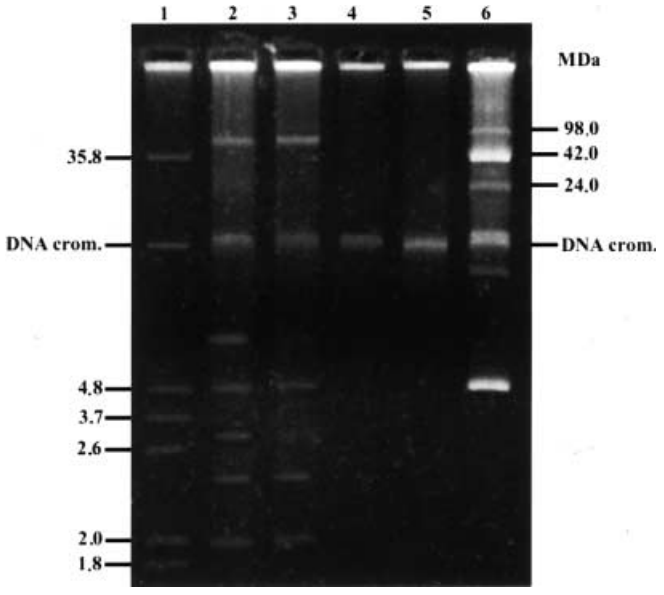

Fig. 1. Plasmid profile of *Escherichia coli* isolates. *Lane 1* Standard plasmids of *E. coli* V517, *lane 2* plasmid profile from strain F15, *lane 3* plasmid profile from strain F16, *lane 4* plasmid profile from strain S12, *lane 5* plasmid profile from strain S13, *lane 6* standard plasmids of *E. coli* 39R 861

we could not detect any plasmid in strains S12 and S13. Figure 1 shows the analysis of the plasmid content of these resistant isolates. The plasmids varied greatly in molecular weight, but two distinct profiles were evident from the banding patterns, displaying a difference of only one band – strain F16 lacks the 4.3 MDa plasmid. Note that similar-sized plasmids do not necessarily establish an epidemiological association between samples, but suggest that restriction enzymes should be used for a greater differentiation [14]. These findings nevertheless represent an extremely valuable tool for epidemiological tracking [17]. Threlfall et al. [19] grouped *Salmonella* Enteritidis strains according to plasmid weight and found plasmids of 3.0, 2.8 and 2.0 MDa, similar to those detected in strains F15 and F16 of our study. A major typing limitation is that plasmid may be acquired from another strain in a relatively short time and, according to some authors [9,10], non-conjugative plasmids may spread among marine populations by natural transformation.

Strains S12 and S13 (Fig. 1), which are also resistant to several antibiotics, did not display any plasmids, although they might have been lost during laboratory manipulation. Brown et al. [4] also reported loss of plasmids and suggested that, under laboratory conditions, the absence of antibiotics in the culture media probably enhances plasmid instability.

A general analysis of LT and ST toxins produced by *E. coli* strains displaying multiresistance to antibiotics and containing plasmids leads to the conclusion that seafood vendors must take some kind of sanitary action in order to safeguard consumers' health. Measures limiting the use of antibiotics should also be considered urgent, especially in view of the therapeutic difficulties produced by the increasing need for expensive, last-generation drugs and by the hazard posed by the presence of multiresistant microorganisms in our environment.

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