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Short Communication

In vitro resistance of *Enterobacter cloacae* isolated from fresh seafood to colistin

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Abstract

Introduction: *Enterobacter cloacae* is a clinically important bacterium from the Enterobacteriaceae family. This study evaluated resistance of *E. cloacae* strains from fish (n=14) and shrimp (n=9) to colistin. **Methods:** Biochemical identification and antimicrobial susceptibility tests were carried out in an automated Vitek®2 instrument. **Results:** Colistin resistance was observed in 21.4% and 66.7% of the strains from fish and shrimp, respectively. We observed minimum inhibitory concentrations of ≥16 mg/L and ≤5 mg/L in 8 and 15 of all strains, respectively. **Conclusions:** Fish and shrimp can carry drug-resistant enterobacteria, which can be of clinical interest.

Keywords: Enterobacteriaceae. Vitek. Polymyxin.

Enterobacter cloacae, a member of the Enterobacteriaceae family, is a common bacterium that resides in the gut microbiota in healthy humans[1]. However, this species is also recognized as a major pathogen in nosocomial infections[2], and is associated with food contamination[3].

Recently, this pathogen has emerged as drug-resistant bacterial species; the development of antimicrobial resistance among *Enterobacter* spp., including resistance to extended-spectrum cephalosporins, is of great concern in both human and veterinary medicine[4].

Therefore, isolation of *E. cloacae* strains that are resistant to colistin[5], an antibiotic polypeptide still useful against multi-drug-resistant gram-negative bacteria such as carbapenem-resistant-Enterobacteriaceae[6], is critical. However, some studies have shown that certain *E. cloacae* isolated from foods maybe resistant to this drug.

With the rise in infections due to multidrug-resistant gram-negative bacilli, more emphasis has been placed on colistin[7]. The present study aimed to evaluate resistance to colistin in *E. cloacae* strains isolated from food.

Enterobacter cloacae strains (23) isolated from fish *Oreochromis niloticus* (n=14) and shrimp *Litopenaeus vannamei* (n=9) were used. Fresh seafood samples (n=10) were purchased in the City of Sobral (Ceará State, Brazil). Skin and muscle of each fish (50 g) and whole shrimp samples (50 g) were inoculated in 450 mL lactose broth (Difco, USA), and were incubated at 35°C for 48h. Bacterial growth in lactose broth (10 µL) was plated on brilliant green bile agar (Difco, USA), and was further incubated at 35°C for 24h. Lactose-positive strains were isolated and maintained in skim milk with 20% glycerol. For analysis, all strains were cultivated in tryptone broth soy broth at 35°C for 24 h. The strains were plated on MacConkey Agar (35°C, 24 h), and lactose-positive colonies were used for identification.

The biochemical profiles of the strains used were confirmed by Vitek2 Gram-negative test cards[4] (Biomérieux, France) in an automated Vitek® 2 instrument. All strains used had phenotypic profiles compatible with that of *E. cloacae*.

Antimicrobial susceptibility and minimum inhibitory concentrations were assessed in the automated Vitek® 2 instrument with AST cards (Biomérieux, France)[8].

We observed resistance to colistin in a total of 9/23 (39.1%) isolates - 6/9 (66.7%) from shrimp and 3/14 (21.4%) from fish. Minimum inhibitory concentration (MIC) of ≥16 mg/mL was observed in 8 strains (5 from shrimp and 3 from fish) and an MIC of ≤5 mg/mL was detected in 15 strains (11 from fish and 4 from shrimp). Thus, our results confirmed the presence of colistin-resistant bacteria in animals from aquaculture. The difference between the contamination indices maybe associated with different culture conditions under which the shrimp and fish were maintained.

In the present study, detection of resistant *E. cloacae* strains maybe indicative of indiscriminate use of drugs in the culture of aquatic organisms, since fish and shrimp, the sources of

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bacteria, were obtained from farms in the State of Ceará, Brazil. Antibiotic use in farm animals has been criticized for contributing to antibiotic resistance[9].

Recent increases in antimicrobial resistance have been recognized. A relatively novel method for combating multi-drug resistant bacteria is the use of antimicrobial peptides (AMPs), including colistin, which is currently regarded as the last line of antimicrobial therapy[10].

Pathak and Gopal[11] also detected mesothermic and heterotrophic aerobic bacteria that are resistant to colistin (98%) in fish (catfish *Clarias batrachus*). Resistant bacteria in fish may pose risk to fish fauna and public health. In addition, the presence of colistin-resistant gram-negative bacteria (*Vibrio cholerae*) in shrimp was reported by Dalsgaard et al.[12].

In veterinary medicine, colistin has been used for decades to treat and prevent infectious diseases[13]. The administration of antimicrobials in aquaculture provides selective pressures that promote the growth of multiple resistant bacteria in the cultured fish and shrimp[14].

Our results indicated that enterobacteria resistant to drugs are present in fish and shrimp. These data confirm potential risk to consumer health, and point to the need to establish measures that can control the use of antimicrobials in aquaculture as well as to monitor fish intended for human consumption.

Conflict of interest

The authors declare that there is no conflict of interest.

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