

**TITLE:** BIOFILM IMPACT ON ANTIMICROBIAL SUSCEPTIBILITY OF ENTEROAGGREGATIVE *E. coli* IN PEG-LID SYSTEM

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## **ABSTRACT**

Enteroaggregative *Escherichia coli* (EAEC) is an emergent cause of acute and persistent diarrhea worldwide. Biofilm formation, a remarkable aspect of its pathogenicity, may confers significant resistance to antibiotics and to immune response. Severe and persistent cases require therapeutic intervention with antimicrobials drugs, but the usual susceptibility techniques do not reflect their behavior in the biofilm. Furthermore, the diagnosis of EAEC is not performed in the laboratory routine, making it empirical. This study aimed to determine the minimum inhibitory concentration in biofilm (MBIC) of EAEC isolated from “quilombola” children, all sensitive in planktonic form. MBIC was also determined for *E. coli* ATCC 25922 and for the EAEC042 and EAEC17-2 prototype strains. Susceptibility test were realized from at least 20 EAEC strains with biofilm formed in peg lid system ( $1,5 \times 10^8$  CFU/mL) in DMEM with 0.4% glucose. Twofold dilutions of five antimicrobials in Cation-Adjusted Mueller-Hinton Broth were triplicate tested. The optical density at 650 nm ( $OD_{650}$ ) was measured after biofilm recover by ultrasound, before and after incubation at 37°C for 6 hours. The MBIC was determined as the lowest concentration of drug that result in an  $OD_{650}$  difference at or below  $\leq 10\%$  of the mean of two positive controls well readings. The MBIC assay revealed that: (i) 100% (20/20) of the isolates become resistant (R) to tetracycline, 88.9% (16/20) R/I (intermediate) to ceftriaxone and 80% (14/20), to cefotaxime; (ii) 96.3% (26/27) remained susceptible (S) to ciprofloxacin and 75% (15/20), to tobramycin. Antimicrobial concentration to inhibit biofilm (ratio MBIC / MIC) increased  $>128$  times for tetracycline and cefotaxime and  $> 2,000$  for ceftriaxone. It was for the first time that MBIC for the EAEC prototype strains were determined, and they may serve as reaction control together ATCC 25922. In conclusion, ciprofloxacin showed excellent activity against in biofilm of EAEC followed by tobramycin, suggesting that they may be used in the treatment of diarrhea by the EAEC. Moreover, both the 3rd generation cephalosporins (cefotaxime and ceftriaxone) and also tetracycline, antibiotics listed in therapy for EAEC, should not be prescribed for presenting risk of treatment failure.

**Keywords:** Enteroaggregative *Escherichia coli*; Biofilm; Antimicrobial susceptibility; MBIC.

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