

TITLE: ANTIMICROBIAL SUSCEPTIBILITY TESTING IN ENTEROAGGREGATIVE *ESCHERICHIA COLI* BIOFILM

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ABSTRACT

Biofilm formation is a remarkable aspect of enteroaggregative *E. coli* (EAEC) infection which confers resistance to antimicrobial drugs and immune response. Serious and persistent diarrheic EAEC cases require antimicrobial intervention, which is usually empiric due to inherent limitation on clinical routine diagnosis of this pathotype. Quinolones, ampicillin, tetracycline and sulfamethoxazole/trimethoprim are the recommended antimicrobials. The objective of this study was to investigate the susceptibility of EAEC biofilm to different classes of antimicrobials. Twenty EAEC isolates from diarrheic children, two EAEC prototype strains (042 and 17-2) and ATCC 25922 *E. coli*, all of them sensitive in planktonic form, were tested for Minimal Biofilm Inhibitory Concentration (MBIC) to aminoglycoside, 3rd generation cephalosporin, cephamycin, fenichol, quinolone, tetracycline, penicillin and sulfamethoxazole/trimethoprim. Biofilm was formed in microplate covered with peg-lid in Dulbecco's Modified Eagle's medium with 0.4% glucose from ~10⁶ CFU/mL suspension during 24, 48 and 72 hours. These biofilms were exposed to antimicrobials diluted in Cation-Adjusted Mueller-Hinton broth. After incubation at 37°C for 6 hours the biofilm was recovered by ultrasound in Mueller-Hinton broth, and the optical density at 650 nm was measured. Bacterial viability was determined after incubation with selected antimicrobials using triphenyl tetrazolium chloride and subculture on nutrient agar. The MBIC assay revealed that: (i) 100% (20/20) of the strains become resistant to tetracycline, chloramphenicol and sulfamethoxazole/trimethoprim and 90% (18/20) to ampicillin, ceftriaxone and cefotaxime; (ii) 95% (19/20) remained susceptible to ciprofloxacin, 80% (16/20) to cefoxitin and 75% (15/20) to tobramycin; (iii) there was no significant difference of MBIC among biofilm growth for 24, 48 or 72 hours of biofilm formation. The resistant strains remained viable in all antimicrobial concentrations tested. In conclusion, ciprofloxacin showed excellent activity against EAEC biofilm followed by cefoxitin and tobramycin, suggesting that they may be used in empiric treatment of diarrhea by EAEC. In addition, tetracycline, chloramphenicol and sulfamethoxazole-trimethoprim should not be prescribed for diarrhea by EAEC, even if susceptible in planktonic form, due to the possible risk of therapeutic failure.

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

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