Biofilms associated with bowel necrosis: A newly recognised phenomenon in infants

G Brisighelli, S Cox, A Theron, K Pillay, H Rode

The term biofilm has been used to describe a well-organised microbial community enmeshed in a polymeric, carbohydrate-rich extracellular matrix and adhering to an inanimate or living surface. The organisms within it become highly resistant to antibiotic therapy and to the immune system. The reasons for this are complex and are related to the inability of antibiotics to diffuse through the biofilm, to changes in the organisms resulting from the increasing hypoxia within the biofilm, and to the development of persistent resistant cells thought to be a mechanism of survival by the micro-organisms. Although biofilms have been detected in several mucosal locations, their ability to trigger human disease is still a matter of active investigation. The pathogenic role of biofilms has been established for oral infections, chronic wounds, and all died despite appropriate antibiotics. All specimens showed varying degrees of bowel necrosis and an organising acute peritoneal reaction. In addition, all showed colonies of Gram-negative bacteria within a mucopolysaccharide matrix.

Conclusions. The identification of biofilms in necrotic bowel has raised questions regarding their clinical implications. Further studies are needed to evaluate all resected necrotic bowel for biofilms and the clinical implications of this finding.

A newly recognised phenomenon in infants

The medical records, bacteriological findings and tissue biopsies from three infants with bowel necrosis who subsequently died from sepsis were analysed. Tissue sent for histological evaluation was prepared for light microscopy. Haematoxylin and eosin (H&E), Sandiford and Alcian blue/periodic acid Schiff (ABPAS) stains were performed. Tissue samples were ex-waxed for electron microscopy in one case.

Results. The three patients described all had necrotic bowel at laparotomy, all cultured Klebsiella pneumoniae from peritoneal pus swabs, and all died despite appropriate antibiotics. All specimens showed varying degrees of bowel necrosis and an organising acute peritoneal reaction. In addition, all showed colonies of Gram-negative bacteria within a mucopolysaccharide matrix.

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Background. A biofilm is defined as a collection of organisms attached to a surface and surrounded by a matrix.

Objective. To present three cases in which bowel necrosis coexisted with biofilm.

Methods. The medical records, bacteriological findings and tissue biopsies from three infants with bowel necrosis who subsequently died from sepsis were analysed. Tissue sent for histological evaluation was prepared for light microscopy. Haematoxylin and eosin (H&E), Sandiford and Alcian blue/periodic acid Schiff (ABPAS) stains were performed. Tissue samples were ex-waxed for electron microscopy in one case.

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matrix (ABPAS) (Fig. 2, A, B and C). The bacterial organisms were also present in the subserosal vessels, and similar scattered colonies were noted on the mucosal surface and intramurally. No viral inclusions, ova, parasites or fungal organisms were identified.

Transmission electron microscopy demonstrated the bacilli within a matrix consistent with mucopolysaccharide (Fig. 3).

**Case 2**

A male infant born at 28 weeks’ gestation, birth weight 1 180 g, had a difficult neonatal course including respiratory distress due to hyaline membrane disease and NEC and was readmitted with sepsis, pneumonia, anaemia and oedema. He underwent laparotomy for abdominal distension due to a colonic stricture, and subsequent relook laparotomy showed extensive adhesions, exudative fibrin and multiple large pockets of pus. Necrotic bowel was resected. He remained unstable on inotropic and ventilator support and died 24 hours later with sepsis and multiorgan failure.

Blood culture always remained negative, but a pus swab from the peritoneum revealed an abundant growth of multidrug-resistant *K. pneumoniae*.

Histological examination of the resected bowel, during the second procedure, showed transmural necrosis with an acute inflammatory response. There was adjacent organisation and regenerative epithelium. In addition, numerous colonies of Gram-negative bacilli within a mucoid matrix were present transmurally, highlighted on the Sandiford and ABPAS stains. Focal colonies within the submucosa appeared to be within vessels. No viral inclusions, ova, parasites or fungal organisms (ABPAS) were identified.

**Discussion**

The presence of biofilm formation in necrotic bowel has not been identified previously in infants, although it is well documented in septic patients with implanted medical and surgical devices, central venous and urinary catheters, and dental and chronic wound infections. Although the function of biofilms is unknown, their presence in necrotic bowel must have implications for the pathogenesis of bowel necrosis and for its medical and surgical management.

In our study, after conventional staining of a bowel specimen, excessive protein matrix was identified. The specimen was therefore subjected to biofilm specific staining. This led to further biofilm identification on subsequent specimens of necrotic bowel.

The exact process by which biofilm-associated micro-organisms can cause disease is speculative at present. In 2003, Parsek and Singh, in *Science*, suggested four criteria to define infections caused by biofilms:

- The infecting bacteria are adherent to some substratum or are surface associated.
- Direct examination of infected tissue shows bacteria living in cell clusters, or microcolonies, encased in an extracellular matrix. The matrix may often be composed of bacterial and host components.
- The infection is generally confined to a particular location. Although dissemination may occur, it is a secondary phenomenon.
- The infection is difficult or impossible to eradicate with antibiotics despite the fact that the responsible organisms are susceptible to killing in the planktonic state.

The encapsulated aggregations of organisms, which can form within hours, are highly resistant to antibiotic therapy and to systemic and local defence mechanisms. Biofilm infections are rarely resolved, and tissue adjacent to the biofilm may undergo...
collateral damage by immune complexes and invading neutrophils.[8] The reasons for this are multifactorial, with changes in the microorganism, the development of resistant endotoxin formation and inability of antibiotics to diffuse through the biofilm’s polysaccharide-rich matrix.[3,7]

Although *K. pneumoniae* is present as a saprophyte in the gastrointestinal tract, the identification of intramural *Klebsiella* biofilms has not been published in the English paediatric literature.[5,10] The question of whether *Klebsiella*-associated biofilms were direct aetiological factors in causing bowel necrosis and the death of the patients reported on cannot be proven, but their presence satisfies all four of the Parsek and Singh postulates.[6] We postulate that this sole offending organism shielded from environmental defence mechanisms could have played a part in the unfolding pathogenesis, either as a direct pathogen or promoter thereof.

The identification of *Klebsiella* biofilm in necrotic bowel has created a therapeutic dilemma. In *K. pneumoniae*, many studies have been performed in order to better highlight the mechanisms underlying this resistance, and have demonstrated that limitation of the penetration of antibiotic molecules through the biofilm matrix is not the main reason for the increasing resistance; rather it is the slow growth rate of *Klebsiella* spp. in the centre of the biofilm. In any case, other mechanisms are involved, and further studies are required to elaborate on new concepts in the preventive measures against nosocomial *K. pneumoniae* infections in the future.[11] In chronic surface wounds with biofilms, the latter can be eradicated with topical bioflammacide therapy; this would not be an option for gastrointestinal disease.[12]

**Conclusion**

The identification of *Klebsiella*-associated biofilms in necrotic bowel of three infants in our institution has raised questions regarding the clinical implications of these biofilms. In future, all resected necrotic bowel should be investigated for presence of biofilms, and the clinical implications of a finding evaluated. If the presence of biofilm is confirmed in the majority of patients with bowel necrosis who died, this may represent a major therapeutic challenge.


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