Evaluation of biofilm formation by rabbit *Escherichia coli* biotypes

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ABSTRACT: Evaluation of biofilm formation by rabbit Escherichia coli biotypes. Diarrhoea caused by enteropathogenic *Escherichia* (*E.*) *coli* (EPEC) is considered one of the major problems for rabbit breeding. These strains cause a high mortality rate and substantial economical losses. Various are the typing methods applied to the isolates, both phenotypic and genotypic. In one commonly-used biotyping scheme, *E. coli* isolates are assigned to different biotypes, depending on the fermentation of five sugars. The most frequently identified biotypes in Italian farms are the rhamnose negative, B12 and B14, and the rhamnose positive B28 and B30. Furthermore, the rhamnose negative *E. coli* strains seem to be more virulent. In addition, not all the pathogenetic mechanisms of *E. coli* are known. Recently, different authors have pointed out the role of the biofilm formation as a virulent tract in *E. coli* strains. Various cell surface structures such as curli fimbriae, cellulose, flagellin have been implicated in biofilm formation in *E. coli*, conferring an increased resistance to environmental stresses. 112 strains belonging to different biotypes (B12, B14, B30, B31, B23, B19), isolated from rabbits with enteritis were tested for their ability to form biofilm and components as curli and cellulose, contributing to its formation. The results showed that no strains have expressed the biofilm. Cellulose assay was positive in 29/112 (25.9%) strains. Curli fimbriae were not expressed in all the studied strains.

Kew words: *Escherichia coli*, Biotype, Biofilm formation, Rabbit.

INTRODUCTION – In rabbit-fattening farms, enteritis caused by *E. coli* is the main cause of morbidity and mortality in weaned rabbits. The colonization and the proliferation in distal ileum and caecum is caused by enteropathogenic *E. coli* (EPEC) strains. They cause the disruption of the intestinal mucosa. Indeed, virulence is associated with the ability of the bacterium to adhere to intestinal epithelial cells and to colonize the digestive tract by effacing of the microvilli (Blanco *et al.*, 1996). Jerse *et al.* (1990; 1991 cited by Blanco *et al.*, 1996) have identified a chromosomal gene, *eae*, required for the production of attaching and effacing lesions. The product of the gene is an outer-membrane protein termed intimin. In order to characterize the isolates, *E. coli* strains can be assigned to different biotypes, depending on the fermentation of the following sugars: rhamnose, sucrose, sorbose, dulcitol, and raffinose. In Italian farms the most involved biotypes are represented by B12, B14 (both rhamnose negative, rha-) and B28 and B30 (rhamnose positive, rha+) (Agnoletti *et al.*, 2006). Other biotypes, such as B27 and B31 are not linked to enteropathy. Some authors have highlighted that rha- *E. coli* strains possess a major virulence. However, the knowledge about the pathogenetic mechanism is still not completely understood. In some Enterobacteriaceae

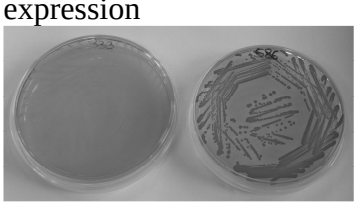
the capability to colonize hosts and survive in the environment is linked to the biofilm formation. Biofilm is defined as a structured community of bacterial cells enclosed in a self-produced polymer matrix that is attached to a surface (Costerton, 1995). Bacteria within biofilms can withstand host immune responses, and are much less susceptible to antibiotics and disinfectants when compared with their planktonic counterparts. Many bacterial surface structures, including curli, flagella, pili, and exopolysaccharide play a role in various aspects of biofilm development (Malcova *et al.*, 2008). In this study we have examined the biofilm formation in 112 field strains of *E. coli*, isolated from rabbits with enteritis. Curli fimbriae and cellulose, extracellular structures contributing to biofilm formation were investigated, too.

MATERIAL AND METHODS – Bacterial strains. 112 strains of *E. coli* isolated from the caecum content of ill rabbits of different ages were used in this study. These strains include biotype B12 (n= 20), B14 (n=29), B30 (n=45), B31 (n=14), B23 (n=2) and B19 (n=2). Biotyping was performed as described by Camguilhem and Milon (1989). Biofilm formation. This test was performed on Congo Red agar plates, in according with Freeman *et al.* (1989). Black colonies on red agar suggest a positive reaction, red ones are typical of a negative reaction (Fig. 1). Curli and cellulose assays. The ability to express curli fimbriae was evaluated by streaking each strain on modified Luria-Bertani (LB) agar plates (without NaCl) of which Congo Red (0.004%) and Coomassie Brilliant Blue G (0.002%) were added. The plates were incubated at 28±1°C for 48-96 hours, aerobically. A positive reaction was indicated by the presence of pink or red colonies (Fig. 2). Cellulose production was determined using the LB-agar plates containing 0.02% Calcofluor. After the overnight incubation at 28±1°C, the plates were checked by UV light illumination (wavelength= 360 nm). A positive result was indicated by fluorescent colonies (Fig. 3) (Hancock *et al.*, 2007).

Figure 1 – Biofilm formation

Positive result on the left side, negative one on the right.

Figure 2 – Curli fimbriae expression



Positive result on the right side and negative on the left.

Figure 3 – Cellulose assay

Positive reaction on the left and negative one on the right.

RESULTS AND CONCLUSIONS – Biofilm formation. All the strains were negative for the production of biofilm. Curli assay showed that no strains were positive. Cellulose production was recovered in 29/112 (25.9%) strains (Table 1). In each biotype, the percentage varies from 5% (biotype B12) to 100% (B23). Among the rha- *E. coli* strains the percentage amounts to 16.3%, while the rha+ *E. coli* strains show a positivity of 33.3% to cellulose formation (Table 2). Both pathogenic and commensal *E. coli* isolates can produce cellulose, curli, or both. In our study, the cellulose production is higher in rha+ strains than in rha- strains. Cellulose is one of the components of the matrix, while curli are important for biofilm development in the initial stages, during the attachment phase. Others polysaccharide components are present (Van Houdt *et al.*, 2005). The biofilm formation represents one of the key factors for protection against phagocytosis and antimicrobial agents. It is also

considered to be a virulence-associated trait. To monitor the ability of the *E. coli* to form biofilm we have used the Congo Red assay. Our tested strains do not form biofilm. As previously described, a plethora of bacterial structures can influence the expression of the slime. Schembri *et al.* (2004) have highlighted that surface structures like capsules can withhold biofilm. Moreover, many structures are prone to phase variation. In this scenario, only a subpopulation of the studied strains could express the biofilm and the components of it (Hancock *et al*., 2007). We therefore have to conduct other tests (e.g. the presence of capsule formation) in order to verify if these structures can interfere with the expression of the biofilm in *E. coli* strains isolated from diseased rabbits. Moreover the presence of the genes codifying for these structures could be evaluated.

Table 1 – Biofilm formation, cellulose and curli fimbriae production in the tested strains

	Biotype	Biofilm formation	Cellulose production	Curli fimbriae
Rha- *E. coli*	**B12**	0/20	1/20	0/20
	B14	0/29	7/29	0/29
Rha+ *E. coli*	**B30**	0/45	11/45	0/45
	B31	0/14	7/14	0/14
	B23	0/2	2/2	0/2
	B19	0/2	1/2	0/2
	TOT	**0/112**	**29/112**	**0/112**

Table 1 – Percentage of cellulose production referred to the different biotypes

	Biotype	Cellulose production	%	
Rha- *E. coli*	**B12**	1/20	5	16.3 %
	B14	7/29	24.1	
Rha+ *E. coli*	**B30**	11/45	24.4	33.3%
	B31	7/14	50	
	B23	2/2	100	
	B19	1/2	50	
	TOT.	**29/112**	**25.9**	

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