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ADHESION OF *YERSINIA ENTEROCOLITICA* TO CHEEK MUCOUS MEMBRANE CELLS OBTAINED FROM HUMANS AND PIGS

Barbara Kot, Katarzyna Wolska
Department of Microbiology, University of Podlasie, Siedlce, Poland

ABSTRACT

Evaluation of adherence of *Y. enterocolitica* strains isolated from humans and pigs to cheek epithelium cells obtained from humans and pigs was the aim of present study. 51 strains of *Yersinia enterocolitica* including 34 isolated from the faeces of people who showed typical symptoms of intestinal yersiniosis and 17 isolated from pigs were used in the study. The all *Y. enterocolitica* strains which were used in the investigation showed the ability of adherence to the cells of cheek epithelium from humans. The cells of *Y. enterocolitica* strains which had *yadA* gene more numerously adhered to the cells of cheek epithelium from humans than the cells of strains which did not have *yadA* gene.

Adhesion of bacterial cells to the human cells of cheek epithelium obtained from four donors and to the cells of cheek epithelium from four pigs was evaluated by using two strains *Y. enterocolitica* which differed in presence of *yadA* gene. The mean values of adherence of *Y. enterocolitica* 97 cells (*yadA*+) both to cheek epithelium cells from humans and from pigs were significantly higher than the mean values of adherence of *Y. enterocolitica* 8 cells (*yadA*-).

Key words: *Yersinia enterocolitica*, adhesion

INTRODUCTION

The interaction between bacteria or bacterial products and host tissues or soluble proteins is crucial during infectious diseases, both for primary adhesion and invasion of the microorganism into the host and for tissue – specific colonization and disease tropism.

A variety of bacteria have been shown to bind host proteins, in particular, the extracellular matrix (EMC) proteins collagen [4, 23], laminin [26], and fibronectin [29].

Yersinia enterocolitica is a common pathogen for both humans and animals. *Y. enterocolitica* is ubiquitous and is most frequently isolated from the environment, e.g., water, soil, and plant surface [16], from food [6], or from pigs, which are an important reservoir for food-borne infections of humans with pathogenic *Y. enterocolitica* 0:3 and 0:9 strains [11]. Enteropathogenic *Yersinia* species can cause different types of diseases; ranging from mild diarrhoea to septicemia; mesenteric lymphadenitis and related diseases that mimic the symptoms of appendicitis are common [2]. The intestinal infection caused by these microorganisms can cause disease sequelae such as reactive arthritis, erythema nodosum, and uveitis [2]. Binding of *Y. enterocolitica* to epithelial cells, to several types of collagen, to fibronectin, and to laminin is YadA - mediated [4, 23, 28]. In addition to having YadA-mediated binding properties, *Y. enterocolitica* possesses other molecules, such as invasin, Ail, and type 3 fimbriae, which mediate binding to a number of targets [17, 27].

Evaluation of adherence of *Y. enterocolitica* strains isolated from humans and pigs to cheek epithelium cells obtained from humans and pigs was the aim of present study.

MATERIALS AND METHODS

Bacterial strains

51 strains of *Yersinia enterocolitica* including 34 isolated from the faeces of people who showed typical symptoms of intestinal yersiniosis and 17 isolated from pigs were used in the study. These strains were previously described [13, 14, 15]. The presence of *yadA* gene in tested strains was determined previously by using PCR method [14, 15].

The cells of cheek epithelium

The cells of cheek epithelium were obtained from 6 healthy humans. The cells of cheek epithelium from pigs were received directly after exanguination of pigs.

The cells of cheek epithelium were suspended in sterile phosphate buffered saline (PBS), pH 7.2 and sedimented by centrifugation (2500 g, 10 min). The cells of cheek epithelium were washed three times with sterile PBS and finally suspened in PBS. The suspension of epithelium cells about 10^5 per ml was used in adherence test.

Adhesion of *Y. enterocolitica* to the cells of cheek epithelium

The tested *Y. enterocolitica* strains were incubated overnight on TSA agar at 25°C. After that the bacterial cells were suspened in PBS pH 7.2. The bacterial density was adjusted turbidimetrically to approximately 10^9 bacteria per ml (A_{600} 0.625) and appropriate concentrations (10^8 per ml) were subsequently obtained by 10-fold dilution in sterile buffer.

1 ml of suspension of bacterial cells (10^8 per ml) was added to 1 ml of suspension of epithelium cells (10^5 per ml) and incubated with shaking for 2 h in 37°C waterbath. The control test was made by incubation of cheek epithelium cells without *Y. enterocolitica* cells in the same conditions.

After incubation, in the aim to remove not adhered bacterial cells the mixture of the epithelium cells and bacteria cells was centrifugated (2500 g, 10 min) and the sediment was washed three times with sterile PBS. 30µl of the sediment was transferred on slide, smeared and then Gram stained. Adherent bacterial cells were counted in relation to 50 of the cheek epithelium cells. The results were expressed as mean number of adherent bacteria to one epithelium cell. Statistical calculation was performed by using Levene test.

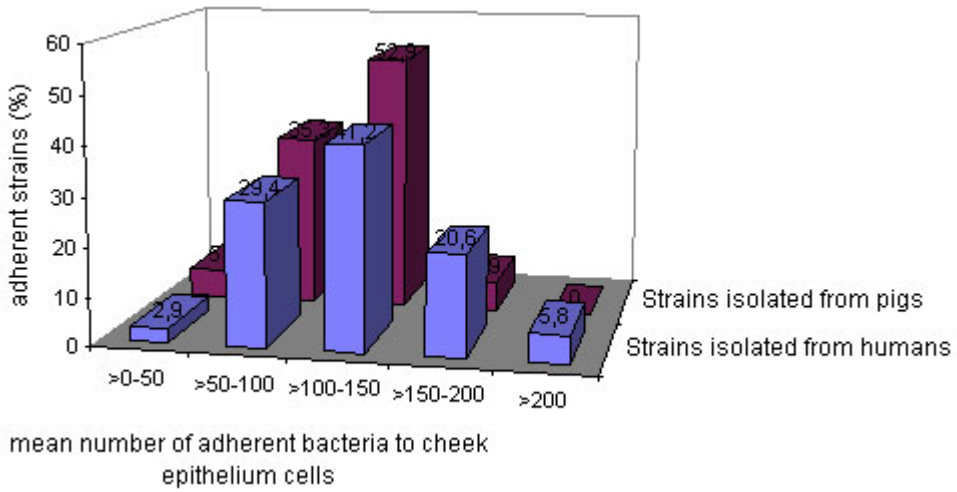
RESULTS

Table 1 shows adherence of *Y. enterocolitica* isolated from humans and pigs to the human cells of cheek epithelium taking into consideration of presence or absence *yadA* gene. The interval >100-150 which determined number of bacterial cells adherent to one epithelium cell was represented by the most number of *Y. enterocolitica* strains both isolated from humans and pigs (Table 1 and Figure 1). The numbers >150-200 and >200 of the bacterial cells adhering to one epithelium cells was also characteristic of some *Y. enterocolitica* strains obtained from humans and one strain from pigs. It was found that these strains had *yad A* gene (Table 1). Whereas the strains of *Y. enterocolitica* with no *yadA* gene present represented the interval >0-50 and >50-100. Two strains of *Y. enterocolitica* which differ in presence of yadA gene were used for further investigation. Adhesion of bacterial cells to the human cells of cheek epithelium obtained from four donors and to the cells of cheek epithelium from four pigs was evaluated. The tested *Y. enterocolitica* strains differed in number of adhering cells. The mean values of the cells of *Y. enterocolitica* 97 strain (*yadA*+) adherence were 158 in the case of the cheek epithelium from humans and 127.82 in the case of the cheek epithelium from pigs. The mean values of adherence of *Y. enterocolitica* 97 cells (*yadA*+) both to cheek epithelium cells from humans and from pigs were significantly higher than the mean values of adherence of *Y. enterocolitica* 8 cells (*yadA*-) which were 89.35 in the case of the cheek epithelium from humans and 50.85 in the case of the cheek epithelium from pigs. Adhesion of *Y. enterocolitica* 8 strain (*yadA*-) to the human cells of cheek epithelium was significantly higher than adhesion to the cells of cheek epithelium from pigs at $p \leq 0.016$.

Table 1. Adhesion of *Y. enterocolitica* to the cells of the cheek epithelium from humans taking into consideration presence of *yadA* gene and origin of strains

Mean number of adherent bacteria cells to cheek epithelium cells	Number of *Y. enterocolitica* strains adherent to cheek epithelium of human cells					
	Strains isolated from humans			Strains isolated from pigs		
	YadA$^+$	*YadA*$^-$	Total	*YadA*$^+$	*YadA*$^-$	Total
>0 - 50	0	1	1	0	1	1
>50 -100	0	10	10	0	6	6
>100 - 150	11	3	14	8	1	9
>150 - 200	7	0	7	1	0	1
>200	2	0	2	0	0	0

Fig. 1. Adhesion of *Y. enterocolitica* to the cells of cheek epithelium from humans taking into consideration origin of strains



DISCUSSION

The ability to adhere to mucosal surfaces is regarded as an important prerequisite for microorganisms which cause disease by invasion of the intestinal mucosa. Enteropathogenic strains of *Y. enterocolitica* are able to adhere to cultured epithelial cells. In contrast, non-pathogenic strains of the same species do not adhere [5]. Cellular penetration of enteropathogenic *Yersinia* species is mediated mainly by invasin, the product of a

chromosomal *inv* gene [21] that initiates entry by binding to mammalian cell receptors belonging to the integrin family [8]. *Y. enterocolitica* also has the chromosomal *ail* gene product, which facilitates adhesion and invasion of bacteria [17]. Invasin is expressed early in infection and is probably important for initiation of gastrointestinal infection, Ail is induced later and is important for extracellular spread [7]. In addition to chromosomally encoded factors, the *Yersinia* virulence plasmid (pYV)-encoded factors also play a role in the bacterium's adhesive properties; pYV–positive *Y. enterocolitica* strains adhere better to intestinal tissue *in vitro* than the corresponding isogenic pYV–negative strains [19]. The pYV plasmid encodes a number of *Yersinia* outer membrane proteins (Yops) which are important virulence determinants. One of these proteins, YadA (*Yersinia* adhesin), is largely responsible for the adhesion of *Y. enterocolitica* to intestinal tissue *in vitro* [20]. Furthermore, YadA has been associated with numerous phenomena: autoagglutination of *Y. enterocolitica* and of *Y. pseudotuberculosis* [25], serum resistance of *Y. enterocolitica* [22], expression of fibrils on the bacterial surface [12] and binding to collagens [4, 23].

The all *Y. enterocolitica* strains which were used in the investigation showed the ability of adherence to the cells of cheek epithelium from humans. The all investigated *Y. enterocolitica* strains had chromosomal *ail* gene, which was determined previously [30]. The cells of *Y. enterocolitica* strains which had *yadA* gene adhered more frequently to the cells of cheek epithelium from humans than the cells of strains which did not have *yadA* gene. The mean value of the cells of *Y. enterocolitica* 97 strain adherence, which had *yadA* gene was statisticaly significantly higher than the mean value of adherence of the cells of *Y. enterocolitica* 8 strain, which did not have *yadA* gene both in case of the cheek epithelium from humans and from pigs. In case of the *Y. enterocolitica* 97 strain significant difference between adherence of the bacteria cells of this strain to the cheek epithelium from humans and pigs was not obtained. Whereas the cells of *Y. enterocolitica* 8 strain which did not have *yadA* gene numerously adhered to the human cells of cheek epithelium. The investigation of other authors concerning the occurrence of *Y. enterocolitica* in slaughter pigs demonstrated that the largest percentage of *Y. enterocolitica* isolation obtained from tongues swabs, following from throat and tonsils. Among isolated strains dominated the strains belonging to 0:3 serotype, biotype 4 [9].

Mouth cavity of slaughter pigs is a main source of *Y. enterocolitica* strains considered as pathogenic for humans [9]. Nesbakken and Kapperud [18] investigated slaughter pigs in Norway, obtained 83.3% of positive samples from mouth cavity of pigs. Andersen [1] obtained this microorganism in 25% tested samples in throat swabs. Quantitative investigations performed by Shiozawa *et al*. [24] showed that larger numbers of *Y. enterocolitica* cells were present in tonsils than in faeces of these same pigs. Jakubczak *et al*. [10] indicated the presence of *Y. enterocolitica* among other things in tonsils of the pigs which were experimentally intravenously and by a stomach infected but he did not indicate *Y. enterocolitica* in gastric contents, duodenum, jejunum, colon or mesenteric lymph nodes. Investigations of Doyle *et al*. [3] and Kapperud [11] indicated that mouth cavity of slaughter pigs is the main source of carcass contamination during exenteration.

CONCLUSIONS

1. The investigated *Y. enterocolitica* strains showed the ability of adherence to the cells of cheek epithelium from humans.
2. The cells of *Y. enterocolitica* strains having yadA gene more numerously adhered to the cells of cheek epithelium from humans than the cells of *Y. enterocolitica* strains without yadA gene.

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Barbara Kot, Katarzyna Wolska
Department of Microbiology
University of Podlasie
ul. B. Prusa 12, 08-110 Siedlce, Poland
e-mail: bkot@ap.siedlce.pl

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