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LS-10-O-2045 Confocal Laser Scanning Microscopy for the detection of intracellular bacterial communities in children

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Urinary tract infection (UTI) is the third cause of medical consultant in childhood. 3-5% of children until the age of 11 are in risk of having UTI. Uropathogenic Escherichia coli (UPEC) is the most common etiological agent, being responsible for 80-90% of cases. Different virulence factors are related with the capability of UPEC of causing UTI and recently it was demonstrated that it can produce intracellular bacterial communities (IBC) in the vesical epithelium in children. Until now, the only technique available for the detection of IBC in urine cells is confocal laser scanning microscopy (CLSM).

The objective of the present work was to determine the presence of intracellular bacteria in children with UTI caused by E. coli, to characterize its virulence attributes and to establish the relationship with the clinical presentation and recurrent UTIs.

The study included 106 children with E. coli UTI assisted in a Children’s Hospital in Uruguay (HP-CHPR) between June-November 2012. Urine samples were analyzed by confocal microscopy for exfoliated urothelial cells with intracellular bacteria. Phylogenetic group and 24 virulence factors of E. coli were determined using multiplex-PCR.

CLSM images allow us to classify at least three different intracellular reservoirs. Intracellular bacteria isolated (IBI) was characterized by the presence of bacteria inside the cell but separated each other (Figure 1). Small intracellular bacterial communities (sIBC) were defined as a group of at least five bacteria (Figure 2) and the presence of a bigger group was classified as intracellular bacterial communities (IBC) (Figure 3). The presence of intracellular bacteria was detected in 37/106 (34.9%) samples, 26 cases as IBC, 11 as intracellular bacteria isolated (IBI). We analyzed 49 medical records: 22 with IBC/IBI and 27 with none IBC/IBI. The presence of IBC/IBI was associated with the absence of certain virulence factors (P pili and siderophores, p0.006 and 0.035 respectively). IBC formation was associated with the presence of lower urinary syndrome and absence of fever (p0.000), not associated with recurrent UTIs. IBI’s presence was associated with recurrent UTI (p0.01).

A new type of UPEC strains with low virulence factors but IBC/IBI producers could be described. The fail of antibiotic therapy could be related with the presence of intracellular bacteria. CLSM should be employed as a gold technique for the detection of IBC in particularly in patients with recurrent UTI. It will also necessary to establish if IBI/sIBC/IBC are different steps from the same phenomena or if they are independent process.
Fig. 1: Intracellular bacteria isolated (IBI). CSLM image; in red uroplakin III from urothelial cell, in green E. coli. The bar represents 7 um.

Fig. 2: Small Intracellular bacteria communities (sIBC). CSLM image; in red uroplakin III from urothelial cell, in green E. coli. The bar represents 7 um.

Fig. 3: Intracellular bacteria communities (IBC). CSLM image; in red uroplakin III from urothelial cell, in green E. coli. The bar represents 7 um.