4: Microorganisms

4.01 Role of intimin and type III secretion system in the ability of an atypical enteropathogenic *Escherichia coli* strain to induce mucus production *in vivo*

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Introduction: Typical and atypical enteropathogenic *Escherichia coli* (EPEC) are major causes of infantile diarrhea in developing countries. The main virulence mechanism of these pathogens is the formation of attaching and effacing lesion (A/E). Two among the various proteins responsible for this lesion are intimin encoded by the *eae* gene and *EscN*, a component of type three secretion system (T3SS), encoded by the *escN* gene. An atypical EPEC strain (aEPEC 3991-1/89) previously isolated in an epidemiological study from a child presenting with diarrheal disease showed the ability of inducing mucus production *in vivo* when tested in the rabbit ileal loop assay. **Objectives:** The objective of this study was to evaluate the role of intimin and the T3SS in the ability of aEPEC 3991-1/89 to induce mucus production *in vivo* in the rabbit ileum. **Methods:** Insertional mutagenesis was used to construct the *eae* and *escN* mutants. To verify the effect of these mutations, we performed the rabbit ileal loop assay, which was analyzed by means of traditional histological and transmission electron microscopy (TEM) techniques. **Results and Discussion:** 1. Individual mutations of the *eae* and *escN* genes abolished completely the ability of aEPEC 3991-1/89 to induce mucus production *in vivo*. 2. Histological and TEM analyses showed that mutation in these genes also abolishes the adherence characteristics of these strains as well as the ability of causing A/E. We conclude that the adherence of aEPEC 3991-1/89 to the enterocyte and/or the formation of A/E lesion are essential processes in triggering mucus production in the *in vivo* assay used. These results corroborate the hypothesis that other pathogenic processes may also be triggered by these initial processes.

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4.02 Biofilm formation among O157:H7 Shiga toxin–producing *Escherichia coli* strains and its correlation with the presence of curli fimbria

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) is an important food-borne pathogen worldwide. Bacterial attachment, colonization, and ability to form biofilm may be important for bacterial persistence in the animal reservoir and food, as well as in food contact surfaces which can serve as a source for biotransfer and cross-contamination of products. **Objectives:** In this study the ability to form biofilm was analyzed in 18 O157:H7 STEC strains isolated from different origins. A correlation between biofilm formation and presence of curli and type 1 fimbria was also investigated. **Methods:** Gene sequences related to curli (*csgA* and *crl*) and type 1 fimbria (*fimH*) were searched by PCR assays. Curli production and the expression of type 1 fimbriae were respectively characterized on Congo red-binding agar plates after 48 h incubation at 28°C, and by hemagglutination assays. Quantitation of biofilm formation was performed by the crystal violet method in 96-well polystyrene microtiter plates at 28°C and 37°C for 48 h. Strains were also analyzed under negative staining by transmission electron microscopy (TEM). **Results and Discussion:** PCR assays showed that *csgA*, *crl* and *fimH* were identified in all O157:H7 strains. Curli production, as judged by characteristic red-colored colonies formed on Congo red-binding agar, was observed in 27.8% (5/18) of the isolates. Phase variant strains, showing both red and white colonies, were identified and re-isolated. Expression of type 1 fimbriae was not observed in any of the strains by hemagglutination assays. The ability to form biofilm at 28°C was only identified in the five O157 curli-producing strains. However, when assays were performed at 37°C, biofilm was observed in 44.4% (8/18) of the isolates, and besides, the five curli-producing strains biofilm occurred in three other curli-negative strains. Quantification of biofilm formation was higher at 28°C when compared to 37°C. The ability to form biofilm was only observed in red colonies among phase variant strains, both at 28°C and 37°C. TEM analysis of red and white colonies of phase variant strains showed the presence of hair-like fimbrial structures similar to curli only in red colonies. The O157 strains able to form biofilm were isolated from cattle. The results obtained suggest that the ability of O157 STEC to form biofilm at 28°C is closely related to curli production; however, at 37°C, in addition to curli, other factors may help in the process of biofilm formation.

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4.03 A newly identified protein of *Leptospira interrogans* mediates binding to laminin

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Introduction: Pathogenic *Leptospira* is the etiological agent of leptospirosis, a life-threatening disease that affects populations worldwide. The search for novel antigens that could be relevant in host-pathogen interactions is being pursued. These antigens have the potential to elicit several activities, including adhesion. **Objectives:** This study focused on a hypothetical predicted lipoprotein of *Leptospira*, encoded by the gene LIC12895, thought to mediate attachment to extracellular matrix (ECM) components. **Methods:** The gene was cloned and expressed in *Escherichia coli* BL21 Star (DE3)pLys using the expression vector pAE. The recombinant protein tagged with N-terminal hexahistidine was purified by metal-charged chromatography and characterized by circular dichroism spectroscopy. The capacity of the protein to mediate attachment to ECM components was evaluated by binding assays. **Results and Discussion:** The leptospiral protein encoded by LIC12895, named Lsa27 (leptospiral surface adhesin, 27 kDa), bound strongly to laminin in a dose-dependent and saturable fashion. Moreover, Lsa27 was recognized by antibodies from serum samples of confirmed leptospirosis specimens in both the initial and the convalescent phases of the disease. Lsa27 is most likely a surface protein of *Leptospira* as revealed in liquid-phase immunofluorescence assays with living organisms. Taken together, these data indicate that this newly identified membrane protein is expressed during natural infection and may play a role in mediating adhesion of *L. interrogans* to its host.

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4.04 Scaling up unencaspulated *Streptococcus pneumoniae* cellular vaccine

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Introduction: *Streptococcus pneumoniae* is responsible for 1.6 millions deaths of children annually worldwide. This pathogen causes otitis media, sinusitis, pneumonia, meningitis and sepsis. There are more than 90 different pneumococcal serotypes, each one corresponding to a capsular polysaccharide. The current vaccines are based on these polysaccharides and require laborious and expensive purification processes to obtain the antigens; as a consequence, the costs of pneumococcal vaccines are high, making it difficult to implement mass vaccination campaigns in developing countries. In order to solve this problem, Malley and collaborators suggested a new vaccine consisting of inactivated whole-cells of unencapsulated *S. pneumoniae*, which would involve lower production costs and provide serotype-independent protection. **Objectives:** To establish a fed-batch fermentation strategy for pneumococcal cultivation and scale-up the production process from 10 to 60 liters. **Methods:** The cultures of the unencapsulated *S. pneumoniae* strain Rx1 Pl^- Al^- kan^R (RM200) have been performed in Bioflo2000 (10L) and Bioflo5000 (60L) bioreactors using enzymatically hydrolyzed soybean meal medium at 2.0% for the inoculum and 0.5% in the bioreactor. A pre-culture was used to inoculate the bioreactor in order to obtain an optical density of ~0.1 at 600 nm (OD_{600}). A fourfold concentrated medium was used for feeding when the OD_{600} reached ~4.5 at 0.5 L/h. The cultures were carried out at 36°C, 150 rpm, 0.5 L/min N_2, 0.1 bar and the pH was controlled at 7.0 by addition of 5 M NaOH. Samples were taken from the bioreactors every 30 min and cell growth was monitored by OD_{600}. After centrifugation of culture broth samples at 20,000 g and 4 °C for 10 min, glucose, lactate and acetate were determined in the supernatant using high-performance liquid chromatography (HPLC, Shimazdu) with an Aminex HPX 87H column (300 x 7.8 mm, BioRad) at 60 °C, and 5 mM H_2SO_4 was used as solvent with a flow rate of 0.6 mL/min. **Results and Discussion:** The fermentation process was reproducible, showing similar results at a 10 L and 60 L scale. Cell growth was satisfactory in both reactors, reaching OD_{600} ~9.5 after 5-6 h. The maximum specific growth rate was 0.96-0.98 h^{-1} in the exponential phase and 0.54-0.59 h^{-1} in the fed-batch phase. The main organic acid produced was lactate, whose concentration reached ~20 g/L at the end of the culture. Although acetate was produced in the batch phase, the concentration dropped from ~4.0 g/L at the beginning of feeding to ~2.7 g/L at the end. More variable data were obtained for glucose consumption, ranging from 3.6 $g/L.h^{-1}$ to 6.2 $g/L.h^{-1}$, which could be explained by the utilization of other carbon sources from the complex medium used. In conclusion, a pneumococcal fed-batch culture was established at 10 L and successfully scaled up to 60 L. Some adjustments should be performed in order to identify the ideal moment for the start of feeding and improve glucose to biomass conversion yield. The developed process would allow vaccine production according to cGMP requirements.

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4.05 Comparison of LD_{50} for botulinum toxin type A in different animal species

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Introduction: *Clostridium botulinum* produces toxins that have been classified into 7 serotypes, A, B, C, D, E, F and G, according to their immunological characteristics. Toxin type A, and sometimes type B, have been used to treat dystonia and various other muscle hyperactivity syndromes in humans. The increase of botulinum toxins in medical use has raised the need for precise analysis of the biological activity contained in the toxin preparations for both clinical use and laboratory investigation. One of the most important parameters that must be observed in pharmaceutical products used in humans is the LD_{50} since it is directly related to safety. **Objectives**: The aim of this study was to compare the value of LD_{50} for botulinum toxin type A in different animal species. **Methods**: According to international convention the fundamental unit (IU) of botulinum toxin biological activity is defined as the LD_{50} for the toxin in a population of mice (1 LD_{50} = 1 IU). We conducted two LD_{50} experiments with guinea pigs and rabbits (25 animals/species). The doses varied between 2 and 4 IU for guinea pigs and 15 and 75 IU for rabbits. The administration of botulinum toxin type A in guinea pigs was performed by intraperitoneal injection, whereas in rabbits by intravenous injection. The LD_{50} was determined using a computerized PROBIT-LOG program. **Results and Discussion**: To compare the LD_{50} between species, the results were converted into IU/kg. The value for guinea pigs was 10.33 ± 1.95 IU/kg, and for rabbits it was 17.31 ± 0.21 IU/kg. Considering that the value for mice is stipulated according international convention at 50 IU/Kg, it is concluded that both these values showed a statistical difference ($p<0.001$). There are significant differences of sensitivity to lethal effects of botulinum toxin type A in rodents, since mice are more resistant than guinea pigs. In the same way, mice are more resistant than rabbits. These data suggest that there are important differences in susceptibility to botulinum toxin type A among animal species, even in the same order (Rodentia) or between different orders (Rodentia x Lagomorpha).

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4.06 Effects of serum on leptospiral binding and on its hemolytic activity

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Introduction: Pathogenic leptospires live both in aquatic environments and inside their hosts. To infect and grow inside hosts, leptospires need nutrients and adhesion capacity to survive in this adverse environment. To obtain iron, an essential nutrient for growth, leptospires secrete hemolytic proteins to release erythrocyte hemoglobin, which contain iron as their prosthetic group. On the other hand, the ability to bind to host molecules, especially to extracellular matrix (ECMs) components, is also a vital feature of these bacteria, since they leave the bloodstream and penetrate several organs, in which they keep multiplying. **Objectives:** The objective of this work was to examine the effects of immune and non-immune serum on leptospiral binding and on its hemolytic activity. **Methods:** Virulent leptospires were cultured in EMJH medium for 10 days at 29° C. They were pelleted and washed three times with PBS. The bacteria were treated with non-immune serum, immune serum or with PBS. Next, each group was allowed to bind to an ECM-like substrate (Matrigel). After several washes and an ELISA-like procedure, the total bacteria bound to the substrate were determined by spectrophotometry. The supernatant of leptospire culture was also treated with non-immune serum, immune serum or with PBS. Afterward, a 5% erythrocyte suspension was added to each group, and total erythrocyte lysis was determined by spectrophotometry. The immune sera were obtained from leptospirosis patients. **Results and Discussion:** Both binding and hemolytic abilities were significantly reduced in the presence of serum, although neither of them was fully inhibited. The reduction levels were not different between groups treated with non-immune and immune serum, indicating that the binding and hemolytic abilities were not significantly affected by the presence of specific antibodies in this condition. The reduction in the hemolysis level was previously described as being a consequence of the competition between the hemolysin targets (the phospholipids and sphingomyelins of the erythrocyte membrane) and the free phospholipids present in serum (especially with the sphingomyelins and phosphatidylcholines). Since leptospires have about 10 proteins that are involved in the hemolytic process, the lack of a full inhibition may be related to the presence of hemolysins with different specializations, such as the SphH protein, described as a pore-forming protein. The addition of an immune serum did not increase the inhibition of leptospire-mediated hemolysis, possibly because not all leptospire hemolysins were recognized by the immune serum. We can hypothesize that a similar process of inhibition may occur in leptospiral binding when in the presence of non-immune and immune serum. Indeed, several proteins have already been described to be involved in leptospiral binding, and some of them can bind to serum components, such as the plasma fibronectin. In conclusion, it seems that the action of some serum components can produce an adverse environment inside hosts, although leptospires seem to be able to overcome it, as a probable consequence of the use of multiple proteins for each function.

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4.07 Purification of a recombinant fragment of pneumococcal surface protein A produced in high cell density culture of *Escherichia coli*

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Introduction: *Streptococcus pneumoniae* is a pathogenic bacterium responsible for millions of deaths in children, elderly and immune-compromised people worldwide. Pneumococcal vaccines have demonstrated a good efficacy, but their coverage is limited due to the large number of serotypes. The polysaccharide vaccines, besides not covering all serotypes, do not protect children under two years old, because the immune response is T-independent. The strategy of Instituto Butantan is to develop a new conjugated vaccine using as carriers pneumococcal surface proteins, which are much more conserved than pneumococcal polysaccharides and would offer a broader coverage. The pneumococcal surface protein A (PspA) was chosen, as it has been demonstrated to be one of the most important virulence factors of *S. pneumoniae*. **Objectives:** To develop an industrial production and purification process of a recombinant fragment of PspA from clade 3 (rfPspA3) in high cell density culture of *E. coli*. **Methods:** *E. coli* BL21(DE3) harboring pET37b+/rf*pspA3* was evaluated for gene expression. Growth kinetics, carbon source consumption, and acetate and protein production were evaluated in shaker flasks and in 5-L bioreactor using high cell density medium (HCD) to compare glucose and glycerol as carbon source. The kinetic parameters were applied to control fed-batch cultures with exponential feeding of the carbon source in order to achieve high cell density. The induction was performed with 0.1 mM IPTG+20 g/L lactose for batch cultures and with 0.5 mM IPTG+20 g/L lactose for fed-batch cultures. The cells were disrupted in a continuous high pressure homogenizer. For clarification, three methods were tested: centrifugation and tangential micro- and ultrafiltration. Two sequences of chromatographic steps were evaluated for purification: anion exchange in Q-Sepharose followed by metal affinity in IMAC-Sepharose loaded with Ni^{+2} and IMAC followed by Q-Sepharose. **Results and Discussion:** Glycerol cultures resulted in less acetate formation (< 1.0 g/L) and lower specific growth rate (0.4 h^{-1}) than glucose cultures (up to 5 g/L acetate and ~0.5h^{-1} specific growth rate). The cell and rfPspA3 production were similar in both glucose and glycerol cultures, reaching ~62 g/L of cell dry weight and ~3 g/L of soluble protein in high cell density fed-batch cultures. Although micro- and ultrafiltration were more effective for clarification than centrifugation, they led to higher loss of rfPspA3 (58% and 29%, respectively). The sequence Q followed by IMAC showed higher yield (77%) and purity (81%) than did the inverse sequence (56% of yield and 76% of purity).

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4.08 Relationship between median paralysis dose (PD_{50}) and median lethal dose (LD_{50}) of botulinum toxin type A in mice

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Introduction: Botulinum toxins are paralytic neurotoxins known for their therapeutic and cosmetic potential. Intramuscular injection of purified botulinum toxin, mainly type A, is the treatment of choice for facial wrinkles and a number of muscle hyperactivity syndromes. The international Unit (IU) of botulinum toxin has been defined as the 50% lethal dose (LD_{50}) in mice which reflects the toxic activity of botulinum toxin but does not reflect its pharmacological properties. On the other hand, regional flaccid paralysis reflects the mechanism of action of botulinum toxin in the clinical setting. **Objective:** This study was performed to compare an assay to assess the paralyzing activity (50% paralysis dose - PD_{50}) with the assay to measure the LD_{50}. **Methods:** Three independent experiments were conducted for lyophilized preparations of 100 IU or 50 IU. In all experiments, the botulinum toxin type A produced at Instituto Butantan was used. For PD_{50} different dilutions of botulinum toxin were injected into the gastrocnemius muscle of NIH mice, whereas for LD_{50} they were injected in NIH mice intraperitoneally. In both experiments the mice were observed for 96 h, and the percentage of paralyzed and dead animals was determined at each dose. The PD_{50} was defined as the inverse of the toxin dilution that caused complete local paralysis in 50% of injected animals, while the LD_{50} was determined as the inverse of the toxin dilution that caused death in 50% of injected animals. Probit analysis was performed to calculate the PD_{50} and LD_{50}. **Results and Discussion:** Botulinum type A toxin preparations were analyzed at different times for up to 180 days after the lyophilization process. During the observation time, LD_{50} ranged from 19.2% (100 IU) to 26.6% (50 IU), while the PD_{50} ranged from 13.9% (100 IU) to 15.2% (50 IU). These results indicate that the PD_{50} activity is more stable than the LD_{50} activity. The more concentrated preparations showed less variation in LD_{50} and PD_{50} during the time studied. The linear regression plots showed high correlation coefficient ($r=0.98$) between LD_{50} and PD_{50} assays.

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4.09 Analysis of biofilm formation of atypical enteropathogenic *Escherichia coli* strains by CFU/cm^2 counting and confocal fluorescence microscopy

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Introduction: Microorganisms can live and proliferate as individual cells swimming freely in the environment, or they possess the capacity to adhere forming biofilms in close association with different types of surfaces and interfaces. This type of formation represents colonization mechanisms. Biofilms confer resistance to some antibiotics in the associated bacteria and it is related to bacterial persistence. Strains of typical EPEC and their isogenic mutants in *bfpA* and *espA* form better biofilms in relation to the wild type, indicating the hypothesis that atypical EPEC strains have a greater capacity for biofilm formation. **Objective**: The aim of this study was to verify the capacity of biofilm formation by atypical EPEC strains isolated from children with diarrhea in Salvador (Bahia) on an abiotic surface (polystyrene) and pre-fixed cellular surface (HEp-2) during prolonged periods of incubation at 37°C. **Methods**: One strain of atypical EPEC, isolated from children with acute diarrhea, of each adhesion pattern (localized adhesion, localized-like adhesion, diffuse adhesion, non adherent, undetermined adhesion and aggregative adhesion) was chosen to carry out the experiments. The test was performed for periods of 6, 12 and 18 days. The strains were tested through the direct method of CFU/cm^2 counting attached to the biofilm after disrupting with Triton X-100. After disruption, serial dilutions were made and plated onto agar Luria-Bertani for counting of CFU/cm^2 and the strains were also visualized with a confocal fluorescence microscope. The bacteria were stained with propidium iodide (appearing red), and the cells (when present) were stained with phalloidin-FITC (appearing green). **Results and Discussion:** Through the analysis with confocal fluorescence microscopy, it was possible to visualize the strains that were capable of forming biofilm in pre-fixed cellular surface and abiotic surface, and some formed better biofilms in pre-fixed cells (HEp-2) and others in polystyrene (abiotic surface). Some strains formed a great amount of biofilm in both surfaces studied; in other words, they were not substrate dependent, at least in the substrates and conditions tested in this work. Other strains of atypical EPEC used as control did not form mature structures of biofilm. By the method of counting CFU/cm^2, the strains showed constant values of CFU/cm^2 in the range of 10^8 UFC/cm^2, and the sample that showed a more highlighted aggregative pattern reached a count of 10^9 CFU/cm^2. The strains of atypical EPEC showed great heterogeneity in relation to biofilm formation. Counting CFU/cm^2 should not be used alone to determine biofilm formation; this should be used together with a qualitative method such as confocal fluorescence microscopy which allows the visualization of biofilm structures.

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4.10 Validation of sterility test in isolator of hepatitis B vaccine (rDNA) (VRHB) produced at Instituto Butantan by means of bacteriostatic and fungistatic effect

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Introduction: Verification of hepatitis B vaccine (rDNA) sterility produced by Instituto Butantan is done by filtering the product with 0.45 μm porosity membrane, rinsing this membrane with a neutralizing fluid and subsequent incubation in fluid thioglycollate and soybean-casein digest media. The current standards require that all operational procedures used in quality control must be validated according Good Laboratory Practices (GLP). **Objectives:** The aim of this study was to determine the sensitivity of membrane filtration methodology applied to bacterial and fungal sterility test in hepatitis B vaccine (rDNA) in isolators and to validate this technique under Microbiological Control Section conditions. **Methods:** This study was performed using three batches of hepatitis B vaccine (rDNA) produced by Instituto Butantan, previously evaluated for thimerosal concentration by spectrophotometric method. These product batches were tested according to standard methodology and membranes were rinsed with Diluent Neutralizing Pharmacopoeic solution, DNP. After transferring the content of the container to be tested to the membrane, an inoculum of a small number of viable ATCC microorganisms (not more than 100 cfu) was added to the final portion of DNP used to rinse the membrane. Fluid thioglycollate medium (LTM) was challenged with *Clostridium sporogenes, Pseudomonas aeruginosa* and *Staphylococcus aureus,* and soybean-casein digest medium (SCM) was challenged with *Aspergillus niger, Bacillus subtilis* and *Candida albicans*. Culture media were incubated for not more than 5 days at 20 °C - 25 °C and 30 °C –35 °C (SCM and LTM, respectively). **Results and Discussion:** Clearly visible growth of all microorganisms was obtained after the incubation period. The methodology applied in isolator to test the bacterial and fungal sterility of hepatitis B vaccine (rDNA) is effective, and thimerosal present in the product formulation as preservative was completely inactivated by rinsing the membrane with DNP solution, allowing the detection of low levels of microbial contamination, ensuring product quality. The sterility test may be carried out without further modifications.

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4.11 Cloning and expression of two predicted surface proteins of *Leptospira interrogans*

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Introduction: Leptospirosis is a zoonosis of global importance that, in recent years, has been considered a major emerging infectious disease. The geographical distribution of leptospirosis is mainly cosmopolitan, where it is associated with inefficiency or lack of infrastructure and poor sanitary conditions. In Brazil, as in other developing countries, most infections occur through contact with water contaminated with rodents' urine, which constitute the main reservoir of these bacteria. In addition, to implement conventional control, new strategies are required to deal with outbreaks. Currently, it is believed that the development of an effective vaccine, with minimum adverse effects, could be an important strategy to prevent the disease. Accordingly, several studies have been conducted to identify and characterize relevant antigens involved in host-pathogen interactions. **Objectives:** Selection, amplification, cloning, expression and purification of two new predicted outer membrane proteins identified in the genome of *L. interrogans* serovar Copenhageni, using *Escherichia coli* as host expression system. **Methods:** Bioinformatics analysis of the sequences encoded by LIC11834 and LIC12253; design of appropriate primers and PCR amplification from genomic DNA; cloning of PCR products in pGEM-T vector; digestion removal of DNA inserts and subcloning in pAE expression vector; sequencing analysis of the cloned inserts; transformation and expression of recombinant proteins in *E. coli* strain BL21 SI; analysis of expression and solubility of the recombinant proteins; purification of the recombinant proteins by metal-affinity chromatography; production of polyclonal antibodies by mouse immunization and immunogenicity tests by ELISA. **Results and Discussion:** After cloning in pAE vector, the proteins could be expressed with the presence of an N-terminal 6xHis tag which makes them suitable for metal-affinity chromatography purification. The recombinant proteins rLIC11834 and rLIC12253 were expressed from induced BL21 (SI) *E. coli* strain in the soluble and insoluble form, respectively. The purification of the proteins was effective, yielding bands with the expected molecular mass of 33 kDa (rLIC11834) and 24 kDa (rLIC12253), as assessed by 12% SDS-PAGE. Mice immunized with the recombinant proteins produced serum with high titers of antibodies against both proteins. These recombinants will be further characterized in other immunoassays to evaluate their participation in leptospiral pathogenesis.

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4.12 Type I fimbriae is the most prevalent fimbrial adhesin in atypical enteropathogenic *Escherichia coli* isolates

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Introduction: Pathogenic *Escherichia coli* strains are classified as diarrheagenic and extra-intestinal *E. coli*, and besides diarrheagenic, they are differentiated into the following pathotypes: enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC) and enteroinvasive *E. coli* (EIEC). Among these pathotypes, EHEC is a subgroup of Shiga toxin-producing *E. coli* (STEC), and EPEC and the EAEC are subdivided into typical and atypical. EPEC is identified by the presence of *eae* and *bfpA* genes which encode the adhesin intimin and the type IV pili BFP, respectively. Both genes are used to classify EPEC into two groups: typical (*eae*+/*bfpA*+) and atypical (*eae*+/*bfpA*-). Current data demonstrate that aEPEC are more prevalent than tEPEC as the main cause of diarrhea in both industrialized and developing countries. Adhesion, an essential first step in bacterial pathogenesis, is mediated by fimbrial adhesins, which are critical for successful *E. coli* colonization in the host's mucosa. **Objective:** In this study, we investigated the prevalence of genes that encode fimbrial components described in some *E. coli* pathotypes among 72 isolates of aEPEC. **Methods:** The PCR technique was employed to search for the presence of the following gene sequences in 72 strains of aEPEC isolated from cases of acute diarrhea: *fimA* and *fimH* which correspond to the type I fimbriae of Enterobacteriaceae; *papA* to P-fimbriae of uropathogenic *E. coli* (UPEC); *aggA, aafA, agg3A* to aggregative adherence fimbriae types I, II and III of EAEC; *pilS* encoding a functional type IV pilus related to AA expression; $lpfa_{O113}$ a long polar fimbriae of Shiga toxin-producing *E. coli* (STEC) of serogroup O113; *sfpA,* a sorbitol-fermenting (SF) EHEC O157:H-; and *lngA* to the longus type IV pilus of ETEC and genes that encode antigens CFA/I, CS1, CS3, CS4 and CS6. The PCR reactions were developed employing specific primers based on published sequences in GenBank. **Results and Discussion:** The *fimA* and *fimH* genes were found in 68 (94.4%) and 70 (97.2%) isolates, respectively. The *pilS* gene was detected in 11 (15.2%) isolates. Regarding the $lpfA_{O113}$ gene sequence, 9 (12.5%) isolates harbored that sequence. The *papA* gene was detected in two (2.7%) isolates and *sfpA* was detected in only in one (1.3%) strain. The other gene sequences were not detected in any isolates. The detection of $lpfa_{O113}$ gene in aEPEC strains corroborates the proposal of a phylogenetic relationship between aEPEC and STEC. The *pilS* gene was described in one particular EAEC strain, where it encoded a functional type IV pilus related to AA expression, and the occurrence of this gene in other *E. coli* pathotypes has yet to be determined. The presence of genes in some strains of aEPEC shows the horizontal transfer of genes between pathotypes of *E. coli*. Despite the fact that the presence of type I fimbriae structures in aEPEC pathogenesis is unclear, the high prevalence of *fimA* and *fimH* genes suggest that they may play a role in the adhesion of aEPEC in the absence of BFP.

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4.13 Screening of *Streptococcus pneumoniae* serotype 14 for capsular polysaccharide production and effect of vitamins on cell growth

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Introduction: The pneumococci are classified into 91 immunologically distinct capsular polysaccharide (CP) types, and their worldwide distribution is variable. CP is the major virulence factor of this microorganism and is the antigen of the available pneumococcal vaccines. In order to produce pneumococcal vaccines, it is essential to: choose serotypes most commonly isolated, select good producer strains, amass knowledge about their metabolism and establish parameters for their cultivation and CP production. In Brazil, the serotype 14 is the most commonly isolated serotype of *S. pneumoniae* in children. **Objectives:** To evaluate the behavior of cell growth and CP production of *S. pneumoniae* serotype 14 strains, selecting the best CP14 producer strain, and to determine which vitamins are essential for growth of the selected strain. **Methods:** Eight strains (731, 322, 334, 366, 941, 1871, 2721 and 5287) were kindly donated by Institute Adolfo Lutz. The inocula were grown from the -70°C stocks in 50 mL of complex medium, in an atmosphere of ~3% CO_2, 37°C and static cultivation. When these cultures were in the exponential phase, the inocula were transferred to flasks containing 500 mL of fresh medium to obtain an initial optical density of ~0.1 at 600 nm (OD_{600}). Cell growth was monitored by OD_{600}, samples were centrifuged and the supernatants were analyzed for CP14 concentration by inhibition ELISA (Malley *et al*, 2006), using CP14 ATCC as standard. To evaluate the vitamins and cofactors that are essential for cell growth, an inoculum of the best CP14 producer strain was prepared as above, then centrifuged, washed with saline and transferred to flasks that contained 50 mL of chemically defined medium - CDM (van de Rijn & Kessler, 1980). The strain was evaluated in complete CDM (control) and in CDM without each vitamin/cofactor to be tested: riboflavin, nicotinamide, pantothenic acid, thiamine, p-aminobenzoic acid, biotin, folic acid, pyridoxamine, pyridoxal, β-NAD, pyridoxamine + pyridoxal and p-aminobenzoic acid + folic acid. All cultures were started with OD_{600} ~0.1 and the growth was monitored by OD_{600}. **Results and Discussion:** All strains analyzed were able to grow in the complex medium; however, 2 strains were withdrawn because their lag phase was >13 h, using the same inoculum ratio as the other strains (0.2% v/v). The six other strains produced CP14 ranging from 74 to 302 mg/L. The highest CP14 concentrations were 302 and 240 mg/L, obtained with strains 1871 and 5287, respectively. Although the highest CP14 concentration was produced by the strain 1871, the strain 5287 was chosen as the best producer because, unlike 1871, it showed a typical stationary phase, a very important feature for bioreactor cultivation. In the assay for essential vitamins/cofactors, only the absence of the vitamins nicotinamide and pantothenic acid affected the cell growth in comparison with the complete CDM, decreasing the maximum OD_{600} by 25% and 73%, respectively. As a result, the number of vitamins/cofactor used in the CDM for the strain 5287 can be reduced from 10 to 2 without decreasing cell growth.

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4.14 Occurrence of *Giardia lamblia* assemblages AII and B, but not AI, in human isolates from São Paulo State, Brazil

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Introduction: Public Health Services in Brazil lack molecular techniques for the investigation of *Giardia lamblia* diarrhea outbreaks, and consequently, the source of infection and routes of transmission cannot be reliably determined. **Objective:** Human *G. lamblia* isolates from various regions of the state of São Paulo, Brazil were genotyped by *gdh* gene sequencing to establish this technique as a tool for investigating *Giardia* diarrheal outbreaks. **Methods:** Cysts of 20 individual isolates obtained after routine stool examination and 7 additional isolates representing four *Giardia* diarrheal outbreaks were partially purified by zinc sulfate flotation, lysed by proteinase K and freeze-thawed; DNA was then extracted with phenol:chloroform. Fragments of ~660 bp of the *gdh* gene were nested-PCR amplified, both strands were sequenced, and the corresponding assemblage determined by alignment with reference sequences. **Results and Discussion:** Individual samples were determined as Assemblage AII (13/20) and B (7/20). One outbreak was caused by Assemblage B and 3 by Assemblage AII. Assemblage AI was not found. These were the first *Giardia lamblia* diarrheal outbreaks investigated by molecular techniques in Brazil, and further samples from these outbreaks will be sequenced. This technique is now available as a tool for investigations carried out by the Epidemiological Surveillance Services.

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4.15 Plasmid profile of atypical enteropathogenic *Escherichia coli*

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Introduction: Diarrhea continues to be one of the most common causes of morbidity and mortality among infants and children, especially in developing countries. Among the bacterial pathogens, diarrheagenic *Escherichia coli* (DEC) is an important agent of endemic and epidemic diarrhea worldwide. The diarrheagenic *E. coli* strains can be classified into six main pathotypes, based on specific virulence properties, clinical features, association with serotypes O:H, epidemiological aspects, and patterns of interaction with cellular strains. Enteropathogenic *Escherichia coli* (EPEC) cause a histopathological lesion known as "attaching and effacing" (A/E). Typical EPEC differ from atypical EPEC by the presence of a plasmid called EPEC adherence factor (EAF) which encodes the bundle-forming pilus (BFP). Atypical EPEC comprise a very heterogeneous group. **Objective:** To study the plasmid profile in a 72-sample atypical EPEC collection. **Methods:** DNA extraction was performed using the Wizard plus midprep DNA purification system, and the results were obtained using 1% agarose gel electrophoresis in Tris-borate-EDTA buffer and ethidium bromide staining. **Results and Discussion:** Besides the fact that atypical EPEC comprise a very heterogeneous group, we found high molecular-weight plasmids ranging from 60 to 80 MDa in most of the strains. We decided to study most specifically plasmids common to strains belonging to the 055:H7 serotype. Using RFLP patterns, we obtained preliminary results indicating that this high molecular-weight plasmid seems to be conserved in these strains. Based on these results, we are working on the characterization of these plasmids to establish their possible involvement in virulence.

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4.16 Cloning and expression of membrane proteins of *Leptospira interrogans*

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Introduction: Leptospirosis is an important global disease of human and veterinary concern, caused by pathogenic spirochaetes of the genus *Leptospira*. Humans are accidental hosts that can be infected by exposure to chronically infected animals and their environment. Bacterial outer membrane proteins (OMPs), particularly those with surface-exposed regions, play a crucial role in the virulence of pathogens and in adaptation to several environmental conditions. Functional genomic studies, including transcription profiles, gene cloning, protein expression and characterization, complement *in silico* analysis and should help our understanding of bacterial pathogenesis. The genome of *L. interrogans* serovar Copenhageni has been sequenced and *in silico* analysis identified more than 200 predicted outer membrane proteins.
Objectives: In this work, our goal was to clone and to study the expression of four genes encoding conserved hypothetical proteins (LIC10411, LIC12891, LIC13305 and LIC11030) with different *Escherichia coli* expression host, inducer concentration and temperature. **Methods:** The genes were amplified by PCR from genomic DNA of *Leptospira interrogans* serovar Copenhageni strain Fiocruz L1-130, using the complementary sequence primers. The genes were cloned into the *E. coli* expression vector pAE at Xho I and Hind III restriction sites. All cloned sequences were confirmed by DNA sequencing in an ABI 3100 automated sequencer. The pAE constructs containing the cloned DNA inserts were employed to transform BL21 SI, BL21 (DE3) Star pLys and BL21 (DE3) *E. coli* strains. Protein expression was analyzed under several conditions, including different concentrations of IPTG /NaCl, protein expression inducers and temperatures. **Results and Discussion:** The choice of predicted proteins was mostly based on their cellular localization. According to the PSORT program, all proteins chosen are predicted to be outer membrane proteins (70% for LIC12891, LIC13305 and LIC11030, and 29.6% for LIC10411). The genes were amplified, without the signal peptide sequence, and the DNA insert cloned and expressed as a full-length protein in *E. coli*. Recombinant proteins were expressed with 6 x His-tag at the N-terminus, facilitating protein purification by metal-affinity chromatography. The recombinant protein LIC10411 was expressed in *E. coli* BL21 (DE3) Star pLys culture with the expected size of 14 kDa. The recombinant protein expressed with 6XHis tag at the N-terminus, was purified by metal chelation chromatography, and an aliquot of each step of the process was analyzed by SDS-PAGE. No expression was observed in cultures containing the pAE LIC12891 construct. Evaluations of the expression of recombinant proteins LIC13305 and LIC11030 are currently underway.

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4.17 Evaluation of the variability of the candidate vaccine PspC (pneumococcal surface protein C) in isolates of *Streptococcus pneumoniae* from University of São Paulo Hospital

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Introduction: *Streptococcus pneumoniae* is part of the normal microflora of the human nasopharynx, being one of the most common causes of respiratory tract infections. The vaccine composed of different capsular polysaccharides (PS) purified from pneumococci has low efficacy in children and the elderly, besides not being able to induce immunological memory. Although the 7-valent PS vaccine conjugated to CRM197 was an advance, its production cost is still a major barrier for its use by the Brazilian public health system. A proposal to increase the vaccine coverage at a low cost consists in the identification of an antigen common to the majority of strains. As a result, protein antigens present in all isolates of pneumococci are being investigated as possible vaccine candidates. PspC (pneumococcal surface protein C) is described as having a role both in the colonization of the nasopharynx and in invasive infection. PspC is highly polymorphic, where it can be divided into 11 groups. Thus, the evaluation of the variability of the antigen in clinical samples is of great importance to determine the ideal vaccine formulation. **Objectives:** The proposal of the present work was to evaluate the variability of *pspC* in Brazilian pneumococcal isolates. We also proposed the expression of variants of PspC and production of anti-PspC antisera to determine the cross-reactivity with different strains of pneumococcus, so that we can assess the potential of vaccination coverage of the expressed proteins. **Methods:** Pneumococcal strains were obtained from the University Hospital of the University of São Paulo. Strains were serotyped by PCR and 13 isolates were chosen based on the serotypes present in the new 13-valent conjugate vaccine (serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F). The complete *pspC* locus was cloned and the gene sequenced for each isolate. BALB/c mice were immunized with two different recombinant PspC variants for the production of antibodies that were used for Western blot analysis. **Results and Discussion:** Of the 13 pneumococcal isolates analyzed, 6 were found to be from group 3, 3 isolates from group 6, 1 isolate from group 5, 1 isolate from group 8 and 1 isolate from group 9. A duplication containing PspC from group 4 and from group 10 was also found. An antiserum raised against PspC3 was able to recognize the majority of pneumococcal extracts by Western blot analysis, showing a broad cross-reactivity. On the other hand, an antiserum raised against PspC8 was able to recognize only the isolate expressing PspC from group 8. These preliminary results suggest that immunization with PspC3 would be a promising strategy capable of inducing antibodies with broad cross-reactivity.

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4.18 Proteomic approach for analysis of fimbrial adhesins of atypical enteropathogenic *Escherichia coli*

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Introduction: Enteropathogenic *Escherichia coli* (EPEC) is considered an emerging bacterial agent associated with child endemic diarrhea worldwide and is classified as typical and atypical. Adherence of typical EPEC to the intestinal mucosa is mediated by the outer membrane protein intimin and BFP fimbria. Since aEPEC is devoid of BFP, another fimbriae other than BFP may be involved in aEPEC adherence to the intestine. **Objectives:** To identify by proteomic analysis the fimbrial structures in aEPEC isolates. **Methods:** Three aEPEC strains with different patterns of adherence (localized-like/LAL, aggregative/AA and diffuse/DA) as well as one nonadherent/NA strain were studied. Strains were grown in TSB and fimbriae were extracted and analyzed by two-dimensional gel electrophoresis (2DE). The MW and pI parameters were used for spot selection in a comparative analysis of the isolate LAL with data from SwissProt database. **Results:** The analysis of the fimbrial extracts of the DA aEPEC by 2DE using a pH 3-10 strip demonstrated that fimbrial proteins were concentrated between pH 4.5-6.5 with PI of 5.09 - 6.21 for proteins with MW of 14-16 kDa. Taking this result into consideration, 2DE gels with pH 4-7 strips were performed with extracts of LAL, AA and NA aEPEC strains and several spots were observed. The comparative study of selected spots of the LAL strain with data from SwissProt showed 11 spots with MW and pI similar to that of fimbrial proteins of non-pathogenic *E. coli* K12, UPEC, tEPEC and ETEC isolated from pig and human. **Discussion:** The comparative analysis of data from the isolate LAL with fimbrial proteins deposited on SwissProt showed that 11 fimbrial structures have MW and pI identical to those of described proteins. In contrast, 16 other selected spots between 14 and 22 kDa had no matching, indicating that these proteins have so far not been identified or characterized. Thus, further mass spectrometry analyses (MALDI-TOF peptide mass fingerprint and/or complementary MS/MS analyses) of the selected spots will be performed and the protein nature of these spots may reveal fimbriae structures involved in aEPEC pathogenesis.

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4.19 Characterization of predicted lipoproteins of *Leptospira interrogans* expressed in *Escherichia coli*

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Introduction: Leptospirosis is a worldwide zoonotic disease caused by pathogenic spirochaetes of the genus *Leptospira*. In urban settings, rodents are the most important carriers of the disease because they continuously shed live leptospires in their urine. Humans can be infected through contact with soil or water contaminated with urine containing leptospires. Since the control of the rodents and sanitation measures are not easily implemented, the development of reliable vaccine is necessary to combat leptospirosis. **Objectives:** The aim of this project was to study three genes that encode predicted lipoproteins selected from the genome sequences of *Leptospira interrogans* serovar Copenhageni. **Methods:** The gene sequences of LIC10258, LIC12880 and LIC12238 were amplified by PCR methodology from genomic DNA of *L. interrogans* serovar Copenhageni and the DNA inserts cloned into the *E. coli* expression vector pAE. The pAE constructs were inserted into BL21 SI *E. coli* strain for protein expression. Subsequently, the recombinant proteins were purified using affinity chromatography. The secondary structure content of the purified proteins was evaluated by circular dichroism (CD) spectroscopy. The cellular localization was performed by liquid-phase immunofluorescence assay (L-IFA). The reactivity of recombinant antigens with human sera of patients diagnosed with leptospirosis was analyzed by ELISA. **Results and Discussion:** Structural integrity of the recombinant proteins was assessed by CD spectroscopy. All proteins showed secondary structures as none of the spectra showed a flat line, characteristic of the denatured non-structured form. Polyclonal serum against rLIC12880 showed positive green fluorescence, which suggests that this protein is surface exposed. The reactivity of the recombinant proteins against paired serum from early and convalescent phase of confirmed leptospirosis patients was evaluated by ELISA. The data showed that all proteins tested were reactive with IgG and IgM antibodies present in the convalescent phase (MAT+). The recombinant protein rLIC12880 did not recognize antibodies in the early phase of the disease (MAT-). However, the rLIC10258 showed a significant frequency of responders (~30%) against IgG antibodies in the early phase. This is important because overall the sensitivity of the tests to diagnose the disease in the first week of illness has been reported to be less than 25%. Therefore, further evaluation of the recombinant protein rLIC10258 with larger samples will indicate its appropriateness for diagnostic purposes.

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4.20 Characterization of a thermoregulated adhesin of *Leptospira interrogans*

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Introduction: Extensive studies aimed at understanding the pathogenesis of *Leptospira* have provided important knowledge about virulence factors. Thus, the expression of genes involved in virulence that are regulated by temperature, as well as the production of proteins capable of interacting with extracellular matrix, are considered critical events during the infection process. **Objectives:** In this study, we set out to characterize a protein, LipL53, from *L. interrogans* previously identified in screening studies to react with a serum sample of an individual diagnosed with leptospirosis (Gamberini et al., 2005). **Methods:** The recombinant protein was expressed as an insoluble form in *E. coli* and refolded by decreasing concentrations of urea during the purification throughout Ni^{2+}-charged chromatography. The presence of the LipL53 transcripts among pathogenic serovars of *Leptospira* and the effect of temperature shift on LipL53 transcription profile were evaluated by RT-PCR. The binding ability of this protein with extracellular matrix components was analyzed by ELISA. **Results and Discussion:** The secondary structure content of recombinant LipL53 as assessed by circular dichroism showed a mixture of β-strands and α-helix. The presence of LipL53 transcripts at 30°C were only detected within the virulent strains. However, upon shifting the attenuated cultures of pathogenic strains from 30 to 37 and 39°C these transcripts could also be observed. The attachment of LipL53 to laminin, collagen I and cellular and plasma fibronectin was specific and dose-dependent. Our results suggest that LipL53 is a novel adhesin of *L. interrogans* that could have an important role in the pathogenesis of the disease.

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4.21 Application of BacT/ALERT 3D system in pulmonary surfactant production control

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Introduction: BacT/ALERT 3D (B/A 3D) system is an automatic equipment used for early detection of bacterial and fungal contamination, and the flasks containing culture media provides nutritional and environmental conditions suitable for microbial growth. This system uses a colorimetric sensor and reflected light to monitor the presence and production of carbon dioxide dissolved in the culture medium, produced as a result of microbial metabolism. Pulmonary surfactant (PS) is a biopharmaceutical extracted from the lungs of pigs used in respiratory distress syndrome which affects newborns. Traditional evaluation of bacterial and fungal sterility of this product is made by direct inoculation (ID) and visual analysis over 14 days. **Objectives:** To implement the B/A 3D system during PS production control by evaluating sensitivity and ability to detect microbial contaminants, reducing the retention time of the product being tested. **Methods:** We used three batches of SP production previously approved by bacterial and fungal sterility tests using traditional methods. We used the Bact/ALERT FA media, with sample volume of 1.0 mL, injected directly into the culture medium and kept under observation for 14 days. At the end of the observation period, there was a new inoculum of ATCC microorganism dilution. Each batch was challenged with an inoculum of less than 100 CFU/mL of the following microbial strains: *Aspergillus niger*, *Bacillus subtilis Candida albicans*, *Clostridium sporogenes, Pseudomonas aeruginosa* and *Staphylococcus aureus*. Sensitivity of culture media was tested with negative control (sterile water) and positive controls (ATCC microorganisms diluted without product in test). **Results and Discussion:** The three lots tested did not reveal the presence of contamination, as with the traditional method. Bact/ALERT FA tested with the product and the inoculum of microorganisms detected ATCC strains with the following average time recovery: *A. niger* 38.2 h, *B. subtilis* 12.7 h. *C. albicans* 35.8 h, *P. aeruginosa* 13.9 h and *S. aureus* 18.3 h. The negative control showed no microbial growth and the positive pure cultures revealed the following recovery times: *A. niger* 41.0 h, *B. subtilis* 12.7 h, *C. albicans* 49.7 h, *P. aeruginosa* 14.4 h and *S. aureus* 20.6 h. The results showed that the B/A 3D system can be used to control the production process, reducing significantly product retention time. Inhibitory components of microbial growth incorporated in the product composition are completely neutralized by BacT/ALERT FA allowing low levels of contamination detection.

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4.22 Quorum sensing in atypical enteropathogenic *Escherichia coli*

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Introduction: Quorum sensing is used to designate a mechanism of gene regulation depending on cell density. The bacteria produce substances that accumulate in growth media, and when these substances reach a threshold concentration, a variety of responses can occur. These molecules are called autoinducers and the phenomena called quorum sensing. At least, four quorum sensing systems were described: two of these systems, which use autoinducer-1 (AI-1) and autoinducer-3 (AI-3), are found in Gram-negative cells while the Gram-positive cells use an autoinducing polypeptide (AIP) system. The fourth system, using autoinducer-2 (AI-2), is found in Gram- positive and Gram-negative cells and might represent a generalized signaling system. It was demonstrated that quorum sensing is involved in type III secretion system regulation, flagellation, and motility in enteropathogenic and enterohemorragic (EPEC and EHEC, respectively) *Escherichia coli* strains. **Objectives:** In this study, we developed the possibility of crosstalk *in vitro* among atypical EPEC and commensal *E. coli*. **Methods:** Pre conditioned medium: bacterial strains were grown to 37°C with aeration until reaching an OD_{600} of 1.0. The growth was centrifuged, and the supernatant was filtered through a 0.22-µm membrane. B-Galactosidase assay: a reporter TEVS232 strain containing the *LEE1:lacZ* fusion was grown in pre conditioned medium until reaching an $OD_{600} \leq 0.2$, and β-galactosidase activity was measured in Miller units. Adherence assays: bacterial strains were grown for 18h in LB medium at 37°C. For the non-induced overnight cultures, 10^5 CFU were added to HEp-2 cells, which were then incubated for 6 h at 37°C with 5% CO_2 , washed with PBS, fixed with methanol and stained with Giemsa stain. PCR: DNA templates for PCR were obtained from overnight *E. coli* cultures that were pelleted, resuspended in 500 µl of sterile deionized water and boiled for 10 min. The gene analyzed in this study was *cif*. Amplified samples were detected by 1% agarose gel electrophoresis in Tris-borate-EDTA buffer and ethidium bromide staining. **Results and Discussion:** It was possible to verify the production of autoinducer AI-3 in the strains tested, and also to quantify their ability of induction. Of the 72 samples studied, 29 tested positive for the gene. The fragment obtained by PCR was submitted to DNA sequencing and showed 98% similarity with the gene described in the literature. Apparently, the inoculation of cell cultures with enterobacteria influences the cellular responses to EPEC strains.

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4.23 Effects of recombinant β subunit phycocyanin in HEp-2 cells

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Introduction: C-phycocyanin (C-PC) is a water-soluble pigment. It is found in some blue-green microalgae such as *Spirulina platensis*, which are used in many countries as dietary supplements. It consists of two subunits, α and β, with molecular masses of 16 and 17 kDa, respectively. One recent study demonstrated that the β subunit of Anabaena C-PC (C-PC) has anti-tumor activity, since the C-PC inhibits cell proliferation and promotes apoptosis in cancer cells. **Objectives:** The aim of this study was the cloning and expression of the β subunit of *A. platensis* C-PC in *Escherichia coli* and to check if the recombinant protein is able to induce apoptosis in the cell line HEp-2. **Methods**: The subunit was cloned in pGEMT- easy plasmid vector producing pTMP-01 plasmid and subcloned in pET28a plasmid vector giving rise to pTMP-02 plasmid. When the cells transformed with the plasmid pTMP-02 reached a growth of OD_{600} between 0.5 and 0.6, they were induced with 1 mM IPTG for 4 h. The gene expression was confirmed by RT-PCR and immunoblotting. The recombinant proteins were purified with Ni-NTA column and were dialyzed and refolded. HEp-2 cells were submitted to incubation with 50 μg of the recombinant protein. The cells were stained with 0.025 % toluidine blue solution in McIlvaine buffer pH 4.0 for 15 min and followed by treatment with 0.05 M aqueous $MgCl_2$ solution for 15 min (critical electrolyte concentration method – CEC). **Results and Discussion:** The β subunit was cloned and expressed in *E. coli* BL21. The CEC method showed apoptosis in cells treated with the recombinant protein. As CEC method is used only as a sign of apoptosis, other tests are needed to quantify apoptosis.

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4.24 Expression of heat-labile (LT) and heat-stable (ST) toxins produced by enterotoxigenic *Escherichia coli* in different enrichment media

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Introduction: Among the diarrhea-associated *Escherichia coli* pathotypes, the enterotoxigenic *Escherichia coli* (ETEC) have been shown to cause up to 10 % of diarrhea in Brazil. These strains produce the enterotoxins, heat-labile (LT) and/or heat-stable toxins (ST) and colonization factors, which allow the organisms to readily colonize the small intestine, and in this way cause diarrhea. Since ETEC can be detected by enterotoxin production, diagnosis must depend upon identifying either LT and/or ST. A prerequisite for successful detection is the production and secretion of the protein in sufficient amounts. There are several conditions and media described to increase toxin production and/or release, including the presence of bile salts or antibiotics such as lincomycin. **Objectives**: Evaluation of ETEC growth and LT/ST production in different broth media. **Methods:** LT- and ST- producing ETEC strain (H10407) was grown at 37 °C (250 rev min^{-1}) for 24 h in five different broth media: Evans, Syncase, tryptic soy broth, *E. coli* (EC) and Dulbecco's Modified Eagle Medium. Bacterial growth was determined at 588 nm, and toxin production was measured in the supernatant by enzyme-linked immunosorbent assay (ELISA). Afterward, the same assay was performed using the adequate medium containing ciprofloxacin or lincomycin. Besides, a collection of 31 ETEC strains was also evaluated in EC broth in the presence or absence of both antibiotics. ST and LT production was detected in the supernatants and in urea-treated pellets of bacterial growth after 7 and 24 h incubation. **Results and Discussion:** The highest level of both toxins in the supernatant was obtained when the H10407 strain was grown in EC broth. Despite the fact that the reference strain showed low growth in the presence of lincomycin, LT and ST production was higher in the medium containing this antibiotic. When the collection of LT-producing ETEC isolates was tested, for some of them LT production increased in the presence of ciprofloxacin, and for other isolates LT production increased in the presence of lincomycin. On the other hand, 85% of the ST-producing ETEC isolates showed considerable increase in toxin when they were grown in the presence of ciprofloxacin. Previous work in our laboratory has demonstrated that EC broth is a suitable medium for Shiga toxin production of STEC isolates and that it is increased with use of ciprofloxacin. In this study, we confirmed that the EC medium can also be employed to enhance ETEC toxin production and that the use of antibiotics could improve toxin production/release. The medium containing lincomycin has been shown to increase LT expression for the H10407 strain, but our results indicate that not all ETEC isolates respond in the same way. Therefore, it is not yet possible to establish a standard condition for LT expression using only lincomycin or ciprofloxacin. Further studies are necessary to optimize LT expression.

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4.25 Analysis of the LEE region of atypical enteropathogenic *Escherichia coli* strains

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Introduction: Enteropathogenic *Escherichia coli* (EPEC) cause the attaching-effacing (A/E) lesion on the intestinal mucosa. A/E is triggered by proteins encoded by the LEE region, which is organized into 5 operons (LEE 1-5). EPEC has been classified as typical and atypical (aEPEC), based on the presence or absence of the EAF plasmid, respectively. In cultured epithelial cells, aEPEC mainly displays the localized-like adherence (LAL) pattern, although the aggregative (AA) or diffuse (DA) adherence may be expressed. **Objectives:** Structural and functional analysis of the LEE region of aEPEC strains. **Methods:** Four aEPEC strains were studied: LAL (O55:H7), DA (O55:H7), AA (O125ac:H6) and nonadherent/NA (O88:HNM). Adherence and capacity to cause A/E (FAS assay) were investigated in HeLa, HEp-2, Caco, T84 and HT29 cells, and Tir phosphorylation in HEp-2 cells. The *tccP (espFu)* gene was searched by PCR. The presence of 31 LEE genes was searched by PCR and slot blot. Transcription of LEE operons was measured by real time PCR (qRT-PCR) and microarray, after the bacterial growth in DMEM (microarray and qRT-PCR) and after bacterial incubation with HeLa cells (qRT-PCR). Expression of intimin, Tir, EspA, EspB and EspD was detected by immunoblotting. **Results and Discussion:** The adherence patterns observed in HEp-2 and HeLa cells were maintained in all cell lines of intestinal origin. The capacity to cause A/E, to phosphorylate Tir and the presence of *tccP* was detected only in the LAL-expressing strain. Among the LEE genes tested by PCR, 11 were not detected in different strains, but these genes were detected by slot-blot. Therefore, the genetic structure of LEE is intact in all aEPEC strains. Transcriptional profiles of LEE as measured by qRT-PCR were analyzed in comparison to the atypical EPEC strain BA320 (LAL/FAS+). LEE 1-5 transcription levels were decreased in the AA, DA and NA strains in both culture conditions (DMEM and HeLa cells), except for LEE 4 (*espA*) which showed higher transcription level in the DA strain in DMEM. Microarray analysis demonstrated that the transcription levels were decreased in comparison to BA320 in the AA, DA and NA strains. All four aEPEC strains studied expressed intimin, Tir, EspA, EspB and EspD. Despite the incapacity to cause A/E, all LEE genes were detected in the AA, DA and NA strains, and the transcription and expression of LEE 1-5 demonstrated that LEE is functional in these strains. The incapacity to cause A/E in these strains could be due to the absence of *perABC* regulators and/or to the inability of Tir phosphorylation and/or the absence of *tccP* expression.

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4.26 The adhesion patterns to epithelial cells of atypical enteropathogenic *Escherichia coli* is modified by secreted proteins that bind to extracellular matrix components

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Introduction: Atypical enteropathogenic *Escherichia coli* (aEPEC) have been a leading cause of childhood diarrhea in developing countries. The main mechanism of atypical EPEC pathogenesis is a lesion called attaching and effacing (A/E), which is characterized by intimate adherence of the bacteria to the intestinal epithelium and destruction of microvillus. Nevertheless, it represents a heterogeneous group and other virulence factors may be involved in atypical EPEC pathogenesis. Previously, we have identified one isolate of atypical EPEC, serotype O26:H11, which secretes proteins that interact with ECM macromolecules. The interactions between pathogenic bacteria and ECM molecules such as fibronectin, laminin and collagen may play an important role in bacterial adherence to and invasion of host cells. Adhesion is critical for successful *E. coli* colonization of the gastrointestinal tract and is mediated by adhesins. **Objective:** The aim of this study was to identify putative adhesins that may contribute to the binding of the isolate of atypical EPEC to extracellular matrix components and to characterize the influence of this interaction in the adhesion to epithelial cells. **Methods:** The supernatant of the atypical EPEC isolate was submitted to a solid phase binding assay with matrigel (a mouse basement membrane composed mainly of laminin, collagen IV and fibronectin), the adhered proteins were stripped from the wells, separated by SDS-PAGE, transferred to nitrocellulose membrane and submitted to immunoblotting assay. **Results and Discussion:** Three major proteins with apparent molecular weights of 107 kDa, 44 kDa and 35 kDa were recognized by the anti-protein polyclonal serum (produced in rabbit immunized with the isolate's supernatant) through immunoblotting assay. In addition, we observed that in the presence of ECM components the isolate clearly changes its adherence pattern to HEp-2 cells, and the number of adhered bacteria to these cells. The atypical EPEC do not share a unique pattern of virulence, suggesting that many virulence factors may contribute to the pathogenesis. The identification of proteins involved in the adhesion with extracellular matrix components in one isolate of this category of diarrheagenic *E. coli* confirms the heterogeneity among the atypical EPEC.

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4.27 Milk, an alternative food source for *Paramecium caudatum* culture

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Introduction: Free-living protozoa are of fundamental importance for the equilibrium of the ecosystem. They are also used in the area of biotechnology for the development of remedies, cosmetics and bio-insecticides. In ecology, apart from contributing to the biodiversity of the microbial fauna, they recycle nutrients and control bacterial populations. The standard methods of culturing protozoa are laborious, time-consuming and costly, since they utilize *Enterobacter aerogenes* as a source of food for the protozoa, and boiled rice to feed the *Enterobacter*. For this reason, the development of quicker and more practical methods to culture protozoa is essential to reduce cost and time and to facilitate their manipulation. **Objective**: The objective of this work was to test an alternative method for the culture of *P. caudatum* using whole cow's milk as food source. **Methods**: For the traditional culture of *Paramecium caudatum*, one grain of boiled rice and 1 ml of sterile mineral water containing 10^6 cells of *Enterobacter aerogenes* were added to 10 ml of sterile mineral water and incubated at 30° C for 120 h. During this period, *Paramecium* growth was determined every 6 h by counting visually the number of cells with the help of a magnifying glass. For the alternative culture of *Paramecium caudatum*, the same procedure as described for the traditional method was used, except that rice and *Enterobacter aerogenes* were replaced by 20 µl of whole milk. **Results and Discussion**: The results showed that *P. caudatum* grows faster on milk than when cultured using the traditional method. Utilizing milk as a source of food in the culture of *P. caudatum* is an easier, less expensive and faster way to grow *Paramecium* ssp.

4.28 Plasmid-encoded toxin (Pet) expressed by enteroaggregative *Escherichia coli* and by atypical enteropathogenic *Escherichia coli*: a comparative study

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Introduction: Among diarrheagenic *Escherichia coli* pathotypes, enteroaggregative *Escherichia coli* (EAEC) and atypical enteropathogenic *Escherichia coli* (aEPEC) have emerged as important pathogens causing diarrheal disease in multiple epidemiologic and clinical settings. It is known that EAEC adhere to the intestinal mucosa and produce enterotoxins and cytotoxins, which lead to a secretory diarrhea and mucosal inflammation. One of the toxins believed to contribute to the pathogenesis of EAEC is the plasmid-encoded toxin (Pet), classified as a serine protease auto-transporter. A role of this protein in mediating enterotoxic activity, is the development of cell exfoliation. EPEC adheres to intestinal epithelial cells and subverts cytoskeletal processes through a histopathological activity on intestinal epithelial cells termed "attaching and effacing" (A/E) lesion. While typical EPEC is homogeneous regarding virulence factors, aEPECs constitute a very heterogeneous group, which can show virulence factors common to other *E. coli* pathotypes. So far, little is known about the repertoire of toxins that aEPEC express. **Objectives:** i) To investigate Pet toxin expression in different aEPEC serotype isolates; ii) To compare Pet toxin expression of aEPEC with EAEC. **Methods**: Cytotoxicity assays in HEp-2 cells were performed with the bacterial culture or the supernatant of the bacterial culture from aEPEC isolate 2275 (O113:H19) *pet/sat*, 3160 (O110:H-) *pet/sat/ Ehly*, 2923 (O34:H6) *pet/sat*, and 2991 (O34:H) *pet/sat,* whose genes were amplified by multiplex PCR, isolate 3170 (O145:H2) PCR negative, and EAEC isolates 91A5; 91A9; 215 A2; 215A3; 215A4; 219A4; 252A1. Purified Pet toxin (200 μg/mL) and 042 (O44:H18), were used as positive controls. **Results and Discussion**: All the isolates tested, except 3170, caused cellular damage when the cells were incubated with the bacterial culture. These cytotoxic effects were neutralized when the bacterial isolates were incubated with PMSF, a serine protease inhibitor, or with the IgG enriched fraction from the anti-Pet polyclonal serum. However, no cytotoxic effects were detected in assays performed with the culture supernatants from the same isolates. The Pet toxin was identified, by immunoblotting, in the culture supernatants from all aEPEC isolates, except 3170. This work demonstrates that several aEPEC isolates may express the Pet toxin, like the EAEC isolates tested; where the *pet* gene was amplified these were able to produce cellular damages such as rounding, elongation and detachment, all typically caused by the Pet toxin. Furthermore, Pet is secreted in the supernatant of the bacteria grown in TSB, but remains inactive in the supernatants from both aEPEC and EAEC. These results demonstrate for the first time that isolates of atypical EPEC may express the Pet toxin like EAEC, attesting to the elevated heterogeneity observed among aEPEC. The lack of Pet toxin activity in the culture medium must be further investigated, but this result suggests that an accessory molecule may be necessary for its activity, which is probably absent or inactive in the supernatant.

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4.29 Analysis of virulence genes in atypical enteropathogenic *Escherichia coli*

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Introduction: Diarrhea continues to be one of the most common causes of morbidity and mortality among infants and children, especially in developing countries. Among the bacterial pathogens, diarrheagenic *Escherichia coli* (DEC) is an important agent of endemic and epidemic diarrhea worldwide. The diarrheagenic *E. coli* strains can be classified into six main pathotypes, based on specific virulence properties, clinical features, association with serotypes O:H, epidemiological aspects, and patterns of interaction with cellular strains. Enteropathogenic *Escherichia coli* (EPEC) cause a histopathological lesion known as "attaching and effacing" (A/E). Typical EPEC differs from atypical EPEC by the presence of a plasmid called EPEC adherence factor (EAF) which encodes the bundle-forming pilus (BFP). Atypical EPEC comprises a very heterogeneous group. **Objective:** We developed multiplex PCR reactions in order to identify virulence genes present in other DEC pathotypes in a 72-sample atypical EPEC collection. **Methods:** DNA templates for PCR were obtained from overnight *E. coli* cultures that were pelleted, resuspended in 500 µl of sterile deionized water and boiled for 10 min. The PCR was developed by combining specific primers for *efa1/lifA*, *pic, pet, astA, hly, sat, toxB, ldaH, ehly1, ehly2, sheA, cdt and saa.* Amplified fragments were detected by 1% agarose gel electrophoresis in Tris-borate-EDTA buffer and ethidium bromide staining. **Results and Discussion:** The results obtained by PCR were confirmed by *Southern blotting* and DNA sequencing. The following were among the most prevalent genes found: *efa* 14.7%, *pic* 1.67%, *pet* 4.17%, *astA* 32.5%, *hly* 0.83%, *sat* 18.83%, *toxB* 2.5%, *ldaH* 6.67%, *ehly*1 5%, *she*A 56.9%, and *cdt* and *saa* 0%. Based on the results, we can affirm that most atypical EPEC strains carry virulence factors common to those of other DEC.

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4.30 Prevalence and antimicrobial profile of uropathogens isolated from the urine of children with urinary tract infection (UTI) from Darcy Vargas Children´s Hospital

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Introduction: The most common bacterial infection recorded in clinical medicine worldwide is urinary tract infection (UTI). *Escherichia coli* is the principal etiological agent isolated in cases of UTI. Recently, an increase has been observed in the antibiotic resistance of pathogens responsible for UTI. This is a serious problem, especially for pediatric patients, to whom treatment is given without the help of an antibiogram analysis. Therefore, it is essential to have prior knowledge of the uropathogens and their antibacterial resistance profile in the population of different regions, since antibiotic resistance of uropathogens varies according to the treatment received. However, few studies have been published about the prevalence and antibiotic resistance of uropathogens especially in children. **Objective:** The objective of this work was to characterize the prevalence of uropathogens, and to determine their resistance to antibiotics in children with clinical diagnosis of UTI. **Methods**: Two hundred and six patients of both sexes between 0 to 15 years of age with clinical diagnosis of UTI from Darcy Vargas Children´s Hospital were analyzed in this work. Bacterial samples isolated from their urine were identified by growth in IAL medium (Instituo Aldolfo Lutz). The antibiogram analysis of the bacterial isolates was determined by the disc diffusion method described by Kirby & Bauer. **Results and Discussion**. The bacterium most prevalent in the urine samples was *Escherichia coli* (54.81%), followed by *Proteus ssp* (18.27 %), *Enterobacter* sp. (5.77%), *Pseudomonas sp*. (6.73%), *Klebsiella* sp. (3.85%), *Enterococcus faecium* (2.88%) and *Candida* spp (6.73%). These results are in accordance with the data obtained from studies performed in different Brazilian age groups, except for the high level of *Candida* spp encountered. The antibiogram results obtained in this work showed that 63.55% of the samples tested are resistant to ampicillin, 37.44% to nitrofurantoin and 29.56% to sulphametrin. Ampicillin is rarely used to treat UTI, but sulfamethoprim is a sulfanomide, a group of antibiotics considered first choice for treatment of UTI. Out of all samples tested, 26% were sensitive to all antibiotics tested, of which 80% were *Escherichia coli.* It is interesting to note that 66.35% of all patients tested were boys whereas only 33.65% were girls. These results are in contrast to the data described in the literature, which indicates that most cases of UTI are recorded in women independent of their age group. These results indicate that the use of antibiotic for treatment of urinary infection can delay the recovery of pediatric patients, which can lead to chronic infection and later renal complications. The results also suggest that the use of antibiotics can induce bacterial resistance in the intestinal commensal bacteria, since 76 % of uropathogens are resistant to at least one of the antibiotics used for urinary infection. In addition the higher number of urinary infection detected in boys can be related to lack of hygiene and the presence of phimosis.

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4.31 New biological function/feature of crotamine

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Introduction: Nowadays, different functional classes of biologically active peptides and toxins isolated from many organisms are known. These compounds can be directly used in medicine or may serve as models for the generation of molecules of medical interest. Crotamine is the most abundant toxin found in the venom of the South American rattlesnake *Crotalus durissus terrificus* and it has been shown to be one of these active peptides with potential pharmaceutical application. This toxin is a strongly basic 42-amino acid residue polypeptide with a molecular weight of about 4.9 kDa. Injection of crotamine in mice induces skeletal muscle spasms, leading to spastic paralysis of the hind limbs, which leads to its inclusion in the small basic myotoxin family. The presence of three disulfide bridges in the crotamine structure gives a high conformational stability to this compound. Interestingly, this same disulfide bridge pattern is also found in antimicrobial peptides from mammals, which usually also show a positively charged surface like crotamine. However, up to now, crotamine was never consistently evaluated as an antimicrobial compound. **Objective:** This study aimed to characterize the antimicrobial activity of crotamine against fungi and bacteria (Gram-negative and positive). **Methods:** The aim of the study was to determine the antimicrobial activity of crotamine purified from the rattlesnake venom, against 10 microorganisms. A colorimetric broth microdilution method was employed for MIC (minimum inhibitory concentration) determination. Microdilution testing was performed according to National Committee for Clinical Laboratory Standards (NCCLS) recommendations (NCCLS document M27-P). Using control growth for comparison, reference microdilution MIC endpoints for crotamine were scored (+) as the lowest concentration at which an absence of growth was observed, and those in which a prominent decrease in turbidity was observed were scored as (++). **Results and Discussion:** The antimicrobial assay demonstrated that crotamine is mainly able to inhibit the growth of fungus, either from reference strains from American Type Culture Collection (ATCC) as well as from clinical isolates. The growth of *Candida krusei, Trichosporon klebahnii, Candida guilliermondii, Candida glabrata, Candida albicans, Candida parapsilosis,* and *Candida tropicalis* was clearly inhibited by crotamine under the conditions used. The antimicrobial activity of crotamine was characterized and the data obtained suggest that this natural compound is a potential candidate for the development of a novel class of antimicrobial compound to treat clinical infections. **CEP:** 1474/07.

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4.32 Validation of sterility test in isolator of adsorbed diphtheria, tetanus and pertussis vaccine (DTP) produced at Instituto Butantan by means of bacteriostatic and fungistatic effect

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Introduction: The verification of sterility of adsorbed diphtheria, tetanus and pertussis vaccine (DTP) produced by Instituto Butantan is done by filtering the product with 0.45 µm porosity membrane, rinsing this membrane with a neutralizing fluid and subsequent incubation in thioglycollate and soybean-casein digest media. The current standards require that all operational procedures used in quality control must be validated according to Good Laboratory Practice (GLP). **Objectives:** The aim of this study was to determine the sensitivity of membrane filtration methodology applied to bacterial and fungal sterility test in DTP vaccines in isolators and to validate this technique under Microbiological Control Section conditions. **Methods:** This study was performed using three batches of DTP vaccine produced by Instituto Butantan, previously evaluated for thimerosal concentration by a spectrophotometric method. These product batches were tested according to standard methods and membranes were rinsing with Diluent Neutralizing Pharmacopoeic liquid, DNP. After transferring the content of the container to be tested to the membrane, an inoculum of a small number of viable ATCC microorganisms (not more than 100 cfu) was added to the final portion of DNP fluid used to rinse the membrane. Fluid thioglycollate medium (LTM) was challenged with *Clostridium sporogenes, Pseudomonas aeruginosa* and *Staphylococcus aureus,* and soybean-casein digest medium (SCM) was challenged with *Aspergillus niger, Bacillus subtilis* and *Candida albicans*. Culture media were incubated for not more than 5 days at 20 °C - 25 °C and 30 °C –35 °C (SCM and FTM, respectively). **Results and Discussion:** Clearly visible growth of all microorganisms was obtained after the incubation period. The methods applied in the isolator to test the bacterial and fungal sterility of DTP vaccine were effective, and thimerosal present in the product formulation as preservative was completely inactivated, raising the membrane with DNP fluid, allowing the detection of low levels of microbial contamination, ensuring product quality. The sterility test may be carried out without further modifications.

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4.33 *Leptospira interrogans* interacts with human plasminogen leading to fibronectin degradation and immune evasion

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Introduction: Leptospirosis is one of the most spread zoonosis worldwide, caused by spirochetes of genus *Leptospira.* Although genomic sequences are available, molecular aspects of pathogenesis, virulence and invasion processes by which these bacteria infect the hosts are poorly characterized. It is well documented that interaction of pathogens with the extracellular matrix (ECM) could play a primary role in the adherence and colonization of host tissues. Indeed, the ability of the leptospires to adhere to ECM has been recently shown. For tissue penetration, proteolytic activity achieved by subversion of host proteases by pathogens, such as plasmin, has been demonstrated to be important in various bacterial infections. Plasmin is a broad-spectrum serine protease component of the fibrinolytic system, composed by the zymogen plasminogen (PLG). **Objectives:** Based on these assertions, we investigated the ability of *Leptospira* to bind PLG and the possible implications in pathogenesis. **Methods:** Bacterial binding to plasmin(ogen) was evaluated by indirect immunofluorescence and by measuring the degradation of specific plasmin substrate. Affinity immunoblotting or modified ELISA was performed to assess the binding of PLG to leptospiral proteins. Human plasma was used to examine immune evasion by modified ELISA. **Results and Discussion:** We demonstrated that leptospires bind purified or plasma PLG *in vitro*, and that binding seems to occur via lysine residues. The binding of PLG to the outer surface of living leptospires was confirmed by confocal microscopy. The PLG-bound bacteria did not exhibit impaired growth and acquired proteolytic activity after addition of exogenous plasmin activator, as evaluated by the degradation of specific plasmin substrate. Plasmin activation was also detected in several species of *Leptospira* but a significantly higher level was observed in a low-passage, virulent strain of *L. interrogans* serovar Copenhageni compared to high-passage non-virulent strain, suggesting the role of this interaction in virulence. Supporting this observation, we demonstrated that several proteins bind PLG in virulent and non-virulent leptospires. We also showed evidence for the participation of fractioned outer membrane proteins in the PLG interaction and showed that neither temperature nor osmolarity shifts in host conditions seemed to influence the binding. Plasmin-coated virulent leptospires were capable of degrading purified ECM fibronectin, an activity that could be important during tissue penetration. We also suggest the role of PLG-binding in the leptospires immune evasion by demonstrating that bacteria bound to plasmin(ogen) are capable of diminishing human C3b complement and human IgG deposition. Our data provide for the first time evidence for the generation of active plasmin on the surface of *Leptospira*, as well as the implications of this phenomenon in infection, tissue penetration and immune evasion. These results give new insights into the understanding of the leptospiral infectious process and molecular pathogenesis of the disease.

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4.34 Antimicrobial susceptibility profiles and biofilm formation of *Staphylococcus aureus* strains

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Introduction: *Staphylococcus aureus* is one of the most important pathogenic bacteria, where it is frequently associated with nosocomial infections. The use of invasive procedures (sera, catheters and surgeries) in patients can also cause an infectious process. *S. aureus* has shown resistance to multiple antimicrobial agents, making it difficult to treat infections. Biofilm is a sessile microbial community characterized by cells that are adhered to a substrate or interface, enclosed in a self-produced polymeric matrix and adherent to an inert or living surface. Biofilm formation is associated with bacterial persistence and resistance to antibiotics. *S. aureus* is capable of biofilm formation, which increases its persistence and boosts its levels of antimicrobial resistance. The importance of studying the biofilm formation in *S. aureus* strains is essential for understanding its role in pathogenesis making it possible to develop therapeutic alternatives. **Objectives:** In this study we evaluated the susceptibility rates of 12 antibiotics and the capacity of biofilm formation on abiotic surfaces in *S. aureus* strains that were isolated from cutaneous abscesses of patients seen at Vital Brazil Hospital and strains of the bacteriological collection of Bacteriology Laboratory of the Butantan Institute. **Methods:** The bacteria were tested by the method of Kirby-Bauer for the antimicrobial susceptibility, utilizing commercially available sensitivity discs and Muller-Hinton agar. The quantitative analysis of the biofilm formation was carried out in polystyrene plates using the crystal violet colorimetric assay, for an incubation period of 24 h at 37°C, after which absorbance was determined at 595 nm in an ELISA plate reader. **Results and Discussion:** The strains of *S. aureus* were sensitive to the following antibiotics: amoxicillin and vancomycin (100%), oxacillin (96%), chloramphenicol (81%), cotrimoxazole (81%), gentamicin (81%) and ciprofloxacin (78 %). Resistance was observed for penicillin (93%), clindamycin and tetracycline (22%) and amikacin (19%). Regarding the antibiotic erythromycin, strains were observed with intermediate sensitivity (41%) and resistant strains (33%). These results demonstrate a variable antimicrobial sensitivity. The high number of resistant strains (mainly for penicillin) shows the necessity of controlling the spread of antibiotic-resistant *S. aureus* strains. Routine monitoring of antibiotic resistance provides data for adequate antibiotic therapy and resistance control of *S. aureus* strains. Based on the colorimetric test with crystal violet, 33% of the strains showed low biofilm formation (OD = 0 – 1.200), 53% showed intermediate formation (OD = 1.201 – 2.400), and only 13% showed high capacity of biofilm formation (OD> 2.401). The majority of samples showed an intermediate level of biofilm formation. This capacity can be explained by the adherence of bacterial cells to a surface and accumulation depending on the growth of bacteria in the multilayer. The capacity of biofilm formation may be important to the existence of bacterial persistence and the resistance to antibiotics in patients.