The gene expression of helicobacter pylori neutrophil-activating protein (HP-NAP), an immunomodulator in allergic asthma: The first case – control study conducted in children living in Istanbul-Turkey

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Background: It was reported that there is an inverse relationship between the presence of H. pylori and asthma and Th2 response may be directed to the Th1 response in asthmatic persons by HP-NAP A. We report the H. pylori quantity in the gut microbiota of children with allergic asthma comparing with that of healthy controls. Additionally, we report the gene expression levels of HP-NAP A.

Methods & Materials: From March 2014 to January 2015 bacterial DNA and RNA were isolated from stool samples of 92 asthmatic children aged from 3 to 8 years and from stool samples of 88 healthy controls. The quantity of H. pylori was determined by Real Time PCR and cDNA synthesis was made from the isolated RNA. The gene expression studies were conducted with HP-NAP A gene (Accession No: U16121.1) and with 16S rRNA gene, primers, probes, cDNAs and Lightcycler 480 Probe master kit, RD in LightCycler 96 instrument. The Cq values obtained from HP-NAP A gene were compared with that of 16S rRNA reference gene and gene expression analyses were performed using delta delta-Ct method.

Results: H. pylori DNA was found negative in stool samples of all 92 asthmatic children and positive in 18(% 20.4) of 88 healthy controls. A statistically significant difference was found between these groups (p< 0.0001, OR = 4.0, 97). The quantity of H. pylori determined by qPCR was higher in 7 of 18 H. pylori positive samples but despite that, in 4 of these 7 samples, the detected HP-NAP A expression levels was low comparing with the levels obtained from 8/11 of the remaining 11 H. pylori positive samples.

Conclusion: Our findings supports the opinion about the presence of an inverse relationship between H. pylori infection and asthma. Additionally, we believe that as well as the presence of HP-NAP A, its expression level plays an important role in the immunomodulation and we can think that its protective effect against asthma is related with the expression level of HP-NAP A rather well the quantity of H. pylori in the fecal microbiota. We believe that more extensive researches with different approaches and different perspectives are needed.

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A case of Actinomyces meyeri empyema: still a challenging entity management

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Background: Actinomycosis is an uncommon chronic infection caused by a group of anaerobic Gram-positive bacilli belonging to the Actinomyces genus, being Actinomyces israelii the most frequently implicated in human diseases. Actinomyces meyeri, in dissimilarity to other species of Actinomyces, is an unusual cause of human actinomycosis, regularly causes pulmonary disease and shows a propensity for hematogenous dissemination. Infection of the respiratory system due to A. meyeri presents with nonspecific symptoms and has no distinctive findings of diagnostic value in radiological tests. Consequently, microbiological and histological examinations are imperative for diagnosis.

Methods & Materials: Here, we report a rare case of empyema caused by A. meyeri.

Results: A 44-year-old male presented with a history of 4 months of dyspnea and chest pain. A large amount of loculated pleural effusion was present on the right side and was documented on radiological studies (Figure 1). A chest tube was inserted and purulent pleural fluid was drained. A. meyeri was isolated in anaerobic cultures of the pleural fluid. The infection was significantly improved in response to treatment with intravenous clindamycin (4800 mg daily) and oral clindamycin (450 mg every 6 hours) for a period of 4 months (Figure 2).

Large loculated effusion in the right hemithorax
**Conclusion:** A review of the English-language literature revealed only six case reports of *A. meyeri* empyema, being the present case the first one reported in Portugal. Four of six patients underwent a surgical procedure, and the duration of antibiotic therapy ranged from 4 to 12 months. In comparison to most of the previous reports, the present case was diagnosed early and was effectively drained with only a chest tube. Additionally, there was no evidence of dissemination and symptoms and radiological findings were rapidly improved, demonstrating that short-term antibiotic treatment may be attempted when the adequate management is promptly instituted according to an early diagnosis and if there is no evidence of dissemination. In conclusion, empyema due to *A. meyeri* is uncommon, and anaerobic culture of pleural fluid plays a main part in the early diagnosis of actinomycosis involving pleura. Although the loculated pleural effusion was large, early diagnosis and successful drainage may abbreviate the duration of treatment.

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### Detection of (hld) gene from staphylococcus epidermidis strains isolated from ICU of Rasul-e Akram hospital, Tehran-Iran

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**Background:** Coagulase negative Staphylococci are the most important hospital pathogens. According to the bacterial virulence factors such as potential ability for biofilm formation and also the emergence of methicillin-resistant strains, delta toxin may lead to the great clinical significant concerns. Delta toxin is encoded by the (hld) gene and a similar system called (agr), which is responsible for regulating.

**Methods & Materials:** In this study, a total of 55 isolates of invasive Staphylococcus epidermidis were collected from different ICU samples of Rasul-e Akram hospital, Tehran, Iran, due to CDC criteria for coagulase negative staphylococci guidelines. All of the isolates were confirmed by API and delta toxin synergistic hemolysis test, finally the prevalence of (hld) gene was estimated by PCR molecular test with specific primers which were designed by primer designer software.

**Results:** Amongst recovered specimens, both blood samples and ear wound infections with (34.5%) and (3.5%) showed the highest and lowest percentage respectively. The synergistic hemolysis was evaluated (58.2%) by phenotypic method, while in genotypic method the frequency of hld gene was determined (74.5%).

**Conclusion:** The prevalence of delta toxin as an important virulence factor in *S. epidermidis* is considered as an essential aspect for determination of invasive strains. In similar studies the mentioned factor has been investigated in NICU while in present study we have compared the both NICU and ICU wards.

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**Single-domain antibody selected from the phage display library neutralizes Escherichia coli endotoxin-induced effects on leukocytes in vitro and in Swiss albino mice**

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**Background:** Lipopolysaccharide (LPS), also called endotoxin, released from G–ve bacteria has a predominant role in sepsis through excessive production of pro-inflammatory mediators. Attempts to design rational therapies against endotoxaemia and sepsis continue. The objective of the present study was to assess the ability of single-domain antibody clones selected from phage display library to neutralize LPS-induced effects on murine and buffalo leukocytes in vitro and in Swiss albino mice.

**Methods & Materials:** Three dAb.HA clones (Cl-18, Cl-23 and Cl-26) originally selected as LPS-binders from the phage display library of LPS-immunized Indian desert camel were sub-cloned in pET303/CT vector/BL21(DE3) host system and expressed as dAb.6×His clones. The clones were purified by Nickel-chelate chromatography, and confirmed by SDS–PAGE and immunoblotting. The nucleotide sequences of the clones were determined.

**Results:** All the dAb clones reacted with both LPS and lipid A in indirect ELISA and exhibited thermo-stability. The affinity constants (Ka) of dAbCl-26, dAbCl-18 and dAbCl-23 for LPS were 4.28 x108/M, 2.18 x108/M and 2.19 x108/M, respectively. Both dAbCl-26 and dAbCl-18 decreased the LPS-induced expression of TNFα, IL-1β and MHC II genes in buffalo leukocytes, and IL-1β, IL-6, CD80, MHC-II and TNFα (only Cl-26) genes in murine macrophages, but dAbCl-23 increased buffalo TNFα and MHC-II, and the murine genes as measured by RT-qPCR. The dAbCl-26 and dAbCl-18 decreased,