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UMP. 637

Neonatal community-acquired infections, when to suspect *Staphylococcus aureus*?

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Background: *Staphylococcus aureus* (SA) including MRSA (methicillin-resistant SA) is infrequent in previously healthy newborns. Since few reports are published about this population in our country, we sought to describe the rate of disease and risk factors associated with SA infection.

Methods & Materials: Unicenter, observational study of neonatal community-acquired (CA) infections. Study period: 01/01/2011 – 12/31/2015 (5 years). Patient charts were reviewed at Neonatal Unit in “Hospital de Niños Ricardo Gutierrez” (Buenos Aires, Argentina). Statistical analysis was performed using Stata v13.

Results: Two hundred seven neonatal CA infections were identified. SA was responsible for 11.59% of them (24/207). All of them were previously healthy term (22) or nearly term (2) infants. Mean age was 16.7 days (SD 7.4 days) and 58.3% (14/24) were male. History of cesarean delivery was present in 52.6% of them (average caesarean delivery in Argentina was 31%). Two patients had family history skin or soft-tissue infections (one of them with concurrent infection). In 16.4% (4/24), a peripheral vascular access had been used previously. Localized skin infection was present in 13/24 (54.17%) cases. Eleven infants (45.8%) had an invasive disease. Most frequent localizations were bone and joint infection, lung and central nervous system. Only one patient had fever of unknown origin at admission.

Fever was more frequent in infants with invasive disease, 64.3% vs 20%; $p=0.04$. White cell count was similar between both groups. C reactive protein (CRP) was performed only to 9 patients with a mean of 1.2 (SD 1.4) in localized infection and 186.4 (SD 177) in invasive disease, $p=0.07$. Almost half of patients (45.8%; 11/24) required surgical drainage. Clinical outcome was favourable with complete resolution in 22/24. Two patients cured with sequelae. None of them died. During 2011–2012 period MRSA rate was 41.7% vs 75% during 2013–2015, $p=0.06$.

Conclusion: *Staphylococcus aureus* is an infrequent cause of neonatal CA infections. It can be present even in infants without any risk factors. Clinical presentation can guide suspicion. Larger studies are needed to evaluate usefulness of CRP at admission and increase of methicillin resistance.

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UMP. 640

Prospective study of timing and pattern of bacteria and viruses in the nasopharyngeal microbiome in a birth cohort

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Background: Potentially pathogenic micro-organisms are present in the nasopharynx of apparently healthy infants and children. This makes it difficult to determine microbial etiology during episodes of upper or lower respiratory tract infection. This prospective cohort study was undertaken to study the timing and pattern of nasopharyngeal microbiome in a birth cohort followed till 2 years of age.

Methods & Materials: A cohort of 100 infants was recruited at birth and followed serially at 3, 6, 9, 12, 15, 21–24 months. At each visit, history and examination was undertaken for upper or lower respiratory tract infection (RTI) during the preceding 14 days, in the infant and family members. Nasopharyngeal aspirate (NPA) was obtained from infants free from RTI symptoms and signs during the preceding 14 days; and processed for bacterial culture and viral multiplex PCR (FTD 21–pathogen kit).

Results: PA obtained at birth from 100 infants yielded organisms in 5 (*Klebsiella*-2, *Enterobacter*-1, *Lecleria*-1, Influenza virus-1). Among asymptomatic infants at the 6 subsequent follow-up visits, bacteria were identified in 31/51 (61%), 41/64 (64%), 39/61 (64%), 40/55 (73%), 39/55 (71%), and 36/46 (78%) respectively. *Moraxella catarrhalis* and *Streptococcus pneumoniae* were detected as early as 3 months and dominated the microbiome at all visits, each comprising 20% of total isolates, followed by Gram-negative bacilli-8%, and *Staphylococcus aureus*-5%. *H. influenzae* was infrequent (1.5%). The number of isolates with potentially pathogenicity at the 7 visits was 3/4 (75%), 23/31 (74%), 32/41 (78%), 28/39 (72%), 32/40 (80%), (79%), and 27/36 (75%) respectively.

Viruses were identified in 11/49 (22%), 17/60 (28%), 22/35 (63%), 43/52 (83%), 39/53 (74%), and 37/46 (80%) samples tested at the six follow-up visits. Among 295 post-birth samples, viruses identified singly or in combination included: Rhinovirus-34%, RSV-23%, Coronavirus-13%, Enterovirus-12%, Adenovirus-11%, Parechovirus-9%, Parainfluenza-8%. At the 6 follow-up visits, viruses associated with pneumonia (RSV, PIV, Influenza, HMPV) were present in 18%, 17%, 72%, 74%, and 78% respectively.



There was no consistency in the bacteria or viruses identified at sequential follow-up visits.

Conclusion: The nasopharyngeal microbiome is established early, is highly diverse and dynamic, comprising several potentially pathogenic bacteria and viruses even when infants are asymptomatic (i.e. free from current/recent RTI).

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UMP. 641

Use of Propidium monoazide for reducing bias in microbiome characterization of Preterm infants at risk of Necrotizing Enterocolitis by DGGE and Sequencing Analysis

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Background: Necrotizing enterocolitis is a deadly disease of preterm infants. Preterm infants with decreased gestational age, born via Caesarean section and deprived of mother milk are at increased risk of acquiring NEC though the exact etiology is not known. Molecular biology techniques have been in use to know the exact causative organism of diseases of unknown etiology. Extracellular DNA may produce bias in molecular studies as that may give signals from dead bacteria. Propidium monoazide is a fluorescent dye that has been used in this study to remove the bias due to extracellular DNA while studying the microbial diversity of Preterm infants stool samples. PMA acts by binding to free DNA after being activated by light and cannot penetrate intact membranes of live cells.

Methods & Materials: After the DNA extraction, PCR amplified 9 clinical stool samples (divide into PMA treated and control samples) were studied by Denaturing gradient gel electrophoresis (DGGE) and next generation sequencing.

Results: Statistically, no significant difference was found between PMA treated and control samples ($P=0.99$). The PLS-DA graph however showed difference in OTUs between positive and control samples. Gel examination also showed variation in bands representing Operational taxonomic units (OTUs) between the two classes of samples. Next generation sequencing data was subsampled by MOTHUR and the difference between Positive and Control samples was not statistically significant. Though, clinically important bacteria as Enterococcus, Escherichia coli and Shigella were increased in proportion in PMA treated samples. Bacteria that might be useful for the infants as Bifidobacterium were decreased in PMA treated samples.

Conclusion: Keeping our initial hypothesis in mind and inferring from our recent data and previous studies, we can propose that NEC is possibly a polymicrobial infection of diverse etiology. PMA treatment has effectively reduced the dead bacteria population according to the criteria of membrane being intact or not. As per results, there is a clearer picture of living bacteria that may be involved possibly in this pathology. However, how in the microenvironment of biofilm these pathogens interact with the normal flora or among themselves may give the exact mechanism of underlying pathogenesis.

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UMP. 642

Application of chromatographic assay and reverse transcriptase polymerase chain reaction for detection of respiratory syncytial virus in pediatric community-acquired lower respiratory tract infections

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Background: Respiratory syncytial virus (RSV) can cause significant morbidity from upper respiratory infections, acute bronchiolitis, and bronchopneumonia to apnea in children. RSV infection can be confirmed using direct fluorescent antibody detection (DFA), chromatographic rapid antigen detection or detection of viral RNA using reverse-transcription polymerase chain reaction (RT-PCR). *The aim of this study was to detect RSV by chromatographic assay and RT-PCR in children with community-acquired lower respiratory tract infections.*

Methods & Materials: In this prospective study, 75 children aged 1 month to 5 years with acute lower respiratory tract infections (LRTIs) were investigated clinically and radiologically. Nasopharyngeal aspirates were obtained on admission for the detection of RSV antigen by using the immunochromatographic test and a RT-PCR to amplify a 287b fragment on RSV G- protein in RSV employing a one step RT-PCR assay. Cases were divided into two groups based on positive or negative findings for RSV and their demographic, clinical and radiological profiles were compared.

Results: RSV infection was positive in 20 (60.6%) children aged <1 year, 13 (39.40%) aged 2-5 years of age group and incidence of RSV across age groups was numerically comparable and the difference was statistically insignificant. Clinical and radiological and features among RSV positive and negative cases were comparable and differences were statistically insignificant. A total of 30 (40%) children were positive for RSV antigen by immunochromatography. RT-PCR for RSV was positive in 33 (44%) children; 30 (90.9%) by RSV antigen; 3 (9.9%) by RT-PCR only. Considering RT-PCR as a diagnostic standard, the sensitivity of RSV antigen by immunochromatography was 90.90%, specificity 100%, positive predictive value 100% and a negative predictive value of 93.3%.

Conclusion: Our data underline the role of respiratory syncytial virus as a major cause of community-acquired lower respiratory tract infections in children aged less than 5 years particularly those aged less than one year.

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