Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

resistant *Staphylococcus aureus* (MRSA), and methicillin-susceptible *S. aureus* (MSSA), respectively, by the MALDI-TOF with correct identification rates of 100%, 97.7% and 93.3%, in comparison with those identified by phenotypic methods. The agreement rate was 75.6% with those determined by the API STAPH when these CoNS isolates were identified into species level by the MALDI-TOF. Seven PVL-positive MRSA isolates were correctly recognized by the MALDI-TOF with 100% accordance with the results determined by real-time PCR. The whole process, from the sample preparation to result analysis, can be completed between 1.5 and 2.5 hours, which greatly shortens the time usually needed for current phenotypic identification.
Conclusion: MALDI-TOF mass spectrometry provides a rapid and relevant system for clinical identification of staphylococci. Detecting PVL protein directly from clinical isolates provides a bacterial identification system that is desirable in clinical diagnostic services.

O484 Development of a multiplex PCR assay for enterotoxigenic *Bacteroides fragilis* and detection of this emerging pathogen in cases of community-acquired diarrhoea in the UK

L.R. Macfarlane-Smith, K.G. Kerr, A.M. Snelling (Bradford, Harrogate, UK)

Objectives: *Bacteroides fragilis* is the Gram-negative anaerobe isolated most often from human infections. Some *B. fragilis* strains produce a 20 kDa enterotoxin (BFT). Enterotoxigenic *B. fragilis* (ETBF) have been isolated from various diarrhoeic animal species and epidemiological studies worldwide note a significant correlation between ETBF and human diarrhoeal disease, especially in children. The prevalence of ETBF in gastrointestinal diseases in the UK, however, has not been investigated, partly due to the lack of a simple identification test that can be used in diagnostic laboratories. The aim of this study was to develop a sensitive and specific multiplex PCR assay for the detection of ETBF directly from faeces, and to investigate if ETBF are associated with cases of community-acquired diarrhoea in the UK.
Methods: Primers were designed from published enterotoxin sequences to amplify 416 bp of bft, and multiplexed with primers amplifying 293 bp of nanH (+ve control for presence of *B. fragilis*). The assay was validated against 136 strains from 52 species. DNA was extracted from stools using the QIAamp DNA stool mini kit (Qiagen). Spiking experiments were used to determine sensitivity of the assay. Stool was obtained from 193 cases of community-acquired diarrhoea, selected on the basis that no other bacterial pathogen had been identified. Where bft was detected in DNA extracts, a second novel multiplex PCR assay was used to determine which of the 3 bft isoforms was present.
Results: The PCR assay for ETBF was found to be 100% specific and had a detection limit of 10^4 cfu/g stool. 13% (25/193) of the diarrhoea samples gave a positive result for bft and nanH. The predominant isoform in the ETBF +ve samples was bft-1 (n = 20, 80%). Three samples had bft-2 and 1 had bft-3. A diarrhoeal sample from a 1-yr old male yielded PCR amplimers for both bft-1 and bft-2 suggesting carriage of at least 2 different ETBF strains.
Conclusion: The multiplex PCR assay was highly specific for ETBF and allowed detection without the need for culture. This is the first report of ETBF in community-acquired diarrhoea in the UK. The distribution of isoforms in the clinical samples was similar to earlier reports from Europe, but the occurrence of 2 different bft isoforms in a faecal sample has not been described before. The finding of ETBF in a high proportion (13%) of samples for which there was no other bacterial explanation for the diarrhoea merits further investigation of this pathogen.

O485 Recently identified viruses contribute significantly to acute respiratory infections in children

A. Pierangeli, C. Scagnolari, S. Trombetti, P. Di Marco, F. Midulla, C. Moretti, G. Antonelli (Rome, IT)

Objectives: More than half of all episodes of acute respiratory infection (ARI) have none of the pathogen identified in paediatric as well as adult populations. It is likely that the prevalence of viral infection is underestimated because of the large number of respiratory viruses involved in respiratory tract infection. A number of novel respiratory pathogens had been identified since 2001, i.e. human metapneumovirus (hMPV), coronaviruses NL63 and HKU1, human bocavirus (hBoV); they are not currently diagnosed but could contribute significantly to the burden of ARI. A molecular approach was undertaken to detect nearly all respiratory viruses, including hMPV, NL63, HKU1 and hBoV in hospitalised children suffering from acute respiratory tract infection.
Methods: Most respiratory viruses were detected prospectively in nasal washes from children hospitalised for ARI in a paediatric department at the University "La Sapienza" hospital of Rome. HKU1 and bocavirus were searched for retrospectively, on frozen aliquots of samples. Reverse transcription-PCR assays followed by sequencing of the amplified fragments were undertaken to detect fourteen respiratory viruses: influenza A and B, RSV, hCoV OC43 and 229E, adenovirus, rhinovirus, parainfluenza viruses 1–3, hMPV, NL63, HKU1 and hBoV.
Results: In 103/227 children (45.4%), at least one viral pathogen was identified; 86/227 (37.9%) had an infection with one of the virus investigated; 17/227 (7.5%) had a dual infection, with a total of 120 viruses identified. The most common agent was RSV, followed by rhinovirus and parainfluenza virus 3. Overall, hMPV infections represented about 8% of all viral illness. One NL63 case was detected; no positive to HKU1 was found. Bocaviruses were detected in 12 cases (11% of all virus positive cases), half of which in co-infections. Almost all patients who had hBoV as the sole pathogen had pneumonia; in addition, it was detected in one children hospitalised for bronchiolitis.
Conclusions: Detection of the recently characterised metapneumovirus, coronaviruses NL63 and HKU1, and bocavirus contributed a significant proportion (17.5%) of all positive samples.
This study is a confirming report of NL63 and hBoV circulation in Italy, reported in late 2006. Interestingly, bocavirus was a frequently detected respiratory agent and was associated with clinically important illnesses.

O486 Implementation of a real-time RT-PCR assay to improve diagnostics of dengue virus infections

A. Dumoulin, H.P. Marti, M. Panning, H.H. Hirsch (Basle, CH; Hamburg, DE)

Objectives: Dengue viruses (DENV) are the most common insect-borne viral pathogens diagnosed in travellers returning from tropical zones. The symptoms caused by DENV range from self-limited, flu-like illness, to life-threatening haemorrhagic fever. DENV are immunologically grouped in four serotypes and secondary infection by a different serotype is presumably one of the factors favouring the haemorrhagic presentation. Routine diagnostics rely on the detection of DENV-specific IgM and IgG. However, because of the low antibody titres in the early phase of disease, serology is prone to false negative results. By allowing detection of the virus in this early phase, RT-PCR can close the diagnostic gap. The objective of this study was to improve the quality of DENV diagnostics at the Zentrum für Infektionsdiagnostik (ZID, Basel) by real-time quantitative RT-PCR, for early detection and molecular typing.
Methods: Serological testing of patient samples was performed by using a rapid commercial assay detecting DENV specific IgM and IgG. A quantitative pan-dengue real-time RT-PCR (PAND PCR) protocol was used to detect DENV independently of the subtype. Classification into the four DENV subtypes was achieved using a serotype specific multiplex real-time RT-PCR protocol. Commercial kits for RNA extraction (QIAamp®, Qiagen) and one-step RT-PCR (iScript®, Biorad) were used. Overall, 98 serum samples were analysed with both methods: subset A was a retrospective panel of 25 IgM positive serum samples; subset B consisted of 73 serum samples (22 IgM positive, 51 IgM negative) collected prospectively at the ZID between April and November 2006.
Results: Of 47 IgM positive samples, 17 (36%) were positive by PAND PCR (subset A: 4 from 25, 28%; subset B: 13 from 22, 59%). Of 51 IgM negative samples (all subset B), 6 were positive by PAND PCR (12%). The detected viral load ranged from 255 copies/mL to