S5 - Epidemiology and Clonality of Multidrug-resistant Acinetobacter Baumannii from a Healthcare Region in Hong Kong

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Introduction: Multidrug-resistant *Acinetobacter baumannii* (MDR-AB) is a rapidly emerging nosocomial pathogen. Depending on the definition, 10–30% of all *A. baumannii* isolates in large hospitals are now resistant to three or more antibiotic classes with clinical utility. Serious infections by A. baumannii are usually treated by one of the antipseudomonal carbapenems if isolates retain susceptibility to this antibiotic class. Nonetheless, carbapenem resistance among MDR-AB is increasingly recognised. In some hospitals, one-half to two-thirds of all *A. baumannii* isolates are now carbapenem resistant.

Objectives: In southeast Asia, *A. baumannii* is known to have been endemic in many hospitals for decades and strains producing metallo-β-lactamases and oxacillin (OXA)-type carbapenemases have been reported. In recent years, we have observed increasing numbers of MDR-AB strains in our healthcare region. This study was therefore conducted to assess the epidemiology of MDR-AB and to define the risk factors for their isolation.

Methods: We assessed the risk factors and molecular epidemiology of MDR-AB in Hong Kong. The patients were treated in five hospitals in a healthcare region during 2005–2006. We performed genomic identification by amplified rRNA gene restriction analysis (ARDRA) and investigated the existence of metallo-β-lactamases and the clonality of representative MDR-AB strains by phenotypic and molecular methods. A case-control study was used to investigate risk factors.

Results: Forty-five subjects with MDR-AB were compared with 135 controls (patients with no MDR-AB). In the logistic regression, chronic wound (odds ratio: 29.5, 95% confidence interval: 8.1–107.2; P<0.001) was the only factor independently associated with MDR-AB colonisation or infection. ARDRA identified all 45 MDR-AB as genomic species 2TU. Pulsed-field gel electrophoresis clustered all except two isolates into two clonal types, designated HKU1 and HKU2 with 24 and 19 isolates, respectively. The main features of HKU1 strains were ST26, adeB type XII, positivity for blaOxA-23-like and blaOxA-51-like genes and high level resistance to carbapenems. Most HKU1 strains retained susceptibility to gentamicin, cotrimoxazole and minocycline. By contrast, HKU2 strains exhibited ST22, adeB type II, and were usually positive only for the blaOxA-51-like gene and resistant to gentamicin, cotrimoxazole and minocycline. Both clones were found to have disseminated widely.

Conclusions: Clonal expansion is playing major roles in the increase of MDR-AB in these hospitals in Hong Kong. The findings highlight the need to enhance infection control measures.

S6 - Estimating the Case Fatality Risk of Human Infections with Avian Influenza A (H7N9)

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Background: Characterisation of the severity profile of human infections with influenza viruses of animal origin is a part of pandemic risk assessment, and an important part of the assessment of disease epidemiology. Our objective was to assess the clinical severity of human infections with avian influenza A (H7N9) virus, which emerged in China in early 2013.

Methods: We obtained information about laboratory-confirmed cases of avian influenza A (H7N9) virus infection reported as of 28 May 2013, from an integrated database built by the Chinese Center for Disease Control and Prevention. We estimated the risk of fatality, mechanical ventilation, and admission to the intensive care unit for patients who required hospital admission for medical reasons. We also used information about laboratory-confirmed cases detected through sentinel influenza-like illness surveillance to estimate the symptomatic case fatality risk.

Results: Of 123 patients with laboratory-confirmed avian influenza A (H7N9) virus infection who were admitted to hospital, 37 (30%) had died and 69 (56%) had recovered by 28 May 2013. After we accounted for incomplete data for 17 patients who were still in hospital, we estimated the fatality risk for all ages to be 36% (95% CI: 26%-45%) on admission to hospital. Risks of mechanical ventilation or fatality (69%, 95% CI: 60%-77%) and of admission to an intensive care unit, mechanical ventilation, or fatality (83%, 95% CI: 76%-90%) were high. With assumptions about coverage of the sentinel surveillance network and health-care-seeking behaviour for patients with influenza-like illness associated with influenza A (H7N9) virus infection, and pro-rata extrapolation, we estimated that the symptomatic case fatality risk could be between 160 (95% CI: 63-460) and 2800 (95% CI: 1000-9400) per 100,000 symptomatic cases.

Conclusions: Human infections with avian influenza A (H7N9) virus seem to be less serious than has been previously reported. Many mild cases might already have occurred. Continued vigilance and sustained intensive control efforts are needed to minimise the risk of human infection.

S7 - Protective Efficacy of Poultry Vaccines against Recently Circulating Highly Pathogenic Avian Influenza (HPAI) H5N1 Virus Isolates from Markets and Farms in Hong Kong 2008

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Introduction: Highly pathogenic avian influenza (HPAI) H5N1 remains a major threat to animal and public health. Since 2003, Hong Kong has successfully used poultry vaccination as part of its strategy to minimise this threat within Hong Kong. In mid-2008, an HPAI H5N1 outbreak occurred in a vaccinated poultry farm in Hong Kong.

Aims: a) to compare protective efficacy of different poultry vaccines against the 2008 farm outbreak strain; and b) to assess whether there needs to be a change in the poultry vaccine used in Hong Kong.

Methods: White leghorn chickens were raised in a clean laboratory environment and divided in four groups: a) unvaccinated controls; or vaccinated with b) Nobilis^{R} vaccine (the vaccine in use in Hong Kong); c) Poulvac^{R} vaccine, or d) Harbin Re-5^{R} vaccine, in accordance with the manufacturer's recommendations. Pre-challenge sera were collected at day 63 of age. Then, 9-12 chicken from each group were challenged by infection with either A/chicken/Hong Kong/8852-2/08 (H5N1), a clade 2.3.4 virus isolated from the affected farm or with A/chicken/Hong Kong/782/2009 (H5N1) (clade 2.3.2). Experimental challenge was done with a dose of 10^5 egg-infection-dose$_{50}$ in a volume of 0.5 mL by the intranasal route. Haemagglutination inhibition (HI) tests were used to evaluate the antibody responses in vaccinated chicken post-immunisation.

Results: All three vaccines provided good protection from death and significantly reduced virus shedding following challenge with A/chicken/Hong Kong/782/2009. Only Harbin Re5 vaccine provided protection against challenge with A/chicken/Hong Kong/8852-2/08, the strain associated with the farm outbreak. Sera from vaccinated chickens had lower geometric HI titres against A/chicken/Hong Kong/8825.2/08, as compared to two other clade 2.3.4 and one clade 0 virus. Alignment of amino-acid sequences of the haemagglutinin of A/chicken/Hong Kong/8825.2/08 and the other H5 viruses revealed several mutations which may correlate with loss of vaccine protection.

Conclusions: Our results indicated that some clade 2.3.4 HPAI H5N1 viruses have undergone antigenic changes that allow them to evade immunity from poultry vaccines. The Nobilis^{R} vaccine then in use in Hong Kong did not provide acceptable protection against some circulating H5N1 virus strains. A change in the poultry vaccine being used in Hong Kong needs to be considered. The findings highlighted the need for ongoing surveillance and monitoring of vaccine-induced immunity against currently circulating virus strains by serological tests supplemented where necessary with experimental vaccine challenge studies in chicken.

S8 - Modulatory Effects of Antimicrobials on the Pathogenicity of Community-Acquired Methicillin-Resistant Staphylococcus Aureus (CA-MRSA) in Hong Kong

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Introduction: Expression of virulence determinants, e.g.panton-valentine leukocidin (PVL), phenol-soluble modulins (PSMs) and alpha-haemolysin (hla) plays an important role in CA-MRSA infections and could be triggered by cell-wall-targeting antibiotics to alter infection outcome.

Objectives: (i) To investigate the in-vitro effects of cell-wall-targeting antibiotics (vancomycin, teicoplanin, oxacillin, cefoxitin and imipenem) on the expression of virulence factors, on prevalent CA-MRSA clones (ST30, ST59) in Hong Kong. (ii) To investigate the in-vivo effects of cell-wall-targeting antibiotics on the treatment outcome of CA-MRSA infection using a mouse necrotising pneumonia model.

Methods: Quantitative PCR was used to investigate the effects of five antibiotics on the in-vitro expression of six virulent genes, virulence gene regulator, agr and msrR. The effects of secreted exotoxins in the culture supernatant were examined by a neutrophil lysis /viability assay. In-vitro effects of virulence expression were correlated to outcome and severity in an optimised mouse pneumonia model.

Results: Subinhibitory concentration of vancomycin induced upregulation of agr and hence expression of virulence genes, hla, pvl and psmα-4 in vitro. This correlated to increased mortality and pneumonia severity in an in vivo murine model. Other antibiotics including teicoplanin, oxacillin, and rifampicin did not upregulate agr, nor increased expression of these virulence genes and demonstrated no mortality and pneumonia scores as that of controls in the mouse model.

Conclusions: 1. The expression of virulence regulator, agr and downstream virulence factors in ST30, a prevalent CA-MRSA clone in Hong Kong, could be modulated to different levels by subinhibitory concentrations of antibiotics.
2.Subinhibitory concentration of vancomycin increased expression of virulence regulator, agr and downstream virulence factors, PVL, PSMα4, and alpha-toxins in ST30 CA-MRSA in vitro. The data was supported by increased mortality and pneumonia severity scores in a mouse pneumonia model.
3.The effects of subinhibitory antibiotics in triggering toxin expression on CA-MRSA appear to be clonally-, drug- and concentration-dependent.
4. A number of other agents at subinhibitory concentrations, e.g. oxacillin, modulate and suppress the expression of these virulence determinants. These deserve further investigations.
5. Selection of antibiotics that modulates or minimises release of toxins is a promising approach to improve treatment outcome. The findings will shed further evidence to the choice of antimicrobials in the treatment of CA-MRSA infections.

S9 - An Infection Control Study for Prevention of Exhaled Air Dispersion during Active Resuscitation and Application of Aerosol-generating Procedures

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Rationale: Tracheal intubation, non-invasive ventilation (NIV), tracheotomy and manual ventilation before intubation are aerosol-generating procedures which increased risk of nosocomial transmission of SARS to healthcare workers (Tran K, et al. PLosOne 2012). We have previously studied manual ventilation ± a filter (Chan MT, et al. AJRCCM 2013). Exhaled air dispersion during 1) NIV via helmets or a total face mask; 2) coughing on endotracheal suction and intubation is unknown.

Methods: We examined the exhaled air dispersion in a negative pressure room with 12 air changes/hr during 1) NIV on a human-patient simulator(HPS) reclined at 45° by two different helmets via a ventilator, and a total facemask via a BiPAP device and 2) endotracheal suction and intubation on the HPS in supine position. Coughing bouts were generated by short bursts of oxygen flow at 650, 320, and 220L/min to simulate normal, mild and poor coughing efforts respectively. Exhaled air was marked by intrapulmonary smoke particles, illuminated by laser light sheet, and captured by a video camera for data analysis. Smoke concentration in the plume was estimated from the light scattered by smoke particles. Significant exposure was arbitrarily defined where there was ≥20% of normalised smoke concentration.

Results: During NIV via a helmet with the HPS programmed in mild lung injury, exhaled air leaked through the neck-helmet interface with a radial distance of 150 to 230mm when inspiratory pressure was increased from 12 to 20cmH_2O respectively, while keeping the expiratory pressure at 10cmH_2O. During NIV via another helmet with air cushion around the neck, there was negligible air leakage (Hui DS, et al. ERS 2013). During NIV via a Respironics total facemask, air leaked through the exhalation port to 618 and 812mm when inspiratory pressure was increased from 10 to 18cmH_2O, respectively, with the expiratory pressure at 5cmH_2O.

While performing endotracheal suction on the HPS before intubation, exhaled air leaked through the mouth of the HPS to 396mm when the HPS was making normal coughing efforts. During normal coughing by the HPS while the endotracheal tube was in place but without suction, exhaled air leaked through the endotracheal tube to 340mm whereas no significant leakage was noted during coughing when endotracheal suction was performed simultaneously (Hui DS, et al. ATS 2014).

Conclusions: Helmet with a good seal around the neck may prevent nosocomial infection during NIV. Constant endotracheal suction can reduce exhaled air leakage when a patient coughs with endotracheal tube in place.