Laribacter hongkongensis and Streptococcus sinensis

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Two novel bacterial species, one of which belongs to a novel genus, were discovered from two patients. These two species, *Laribacter hongkongensis*[1] and *Streptococcus sinensis*,[2] were named after Hong Kong and China respectively, in honour of the place where they were discovered.

In the first case, a 54-year old Chinese man was hospitalised because of fever and shortness of breath for 4 days. He had alcoholic cirrhosis complicated by recurrent ascites. Examination showed an oral temperature of 40.0°C, hepatosplenomegaly, ascites, and a right pleural effusion. Chest radiograph and contrast CT scan of the thorax showed a right-sided empyema and collapse-consolidation of the lower lobe of the right lung. The haemoglobin was 10.8 g/dl, total white cell count 13.0×10^9/l, with neutrophil count 11.7×10^9/l, lymphocyte count 0.3×10^9/l, monocyte count 0.8×10^9/l, and platelet count 75×10^9/l. The serum bilirubin was 50 μmol/l, albumin 20 g/l, alkaline phosphatase 95 U/l, alanine aminotransferase 13 U/l, aspartate aminotransferase 37 U/l, and γ-glutamyl transferase 521 U/l. The serum urea and creatinine levels were within normal limits. The random serum glucose was 15.9 mmol/l, prothrombin time 15.1 s, and activated partial thromboplastin time 38.4 s. Blood culture, thoracocentesis and abdominal paracentesis were performed and empirical intravenous cefuroxime and netilmicin were administered. Pleural fluid examination revealed a white cell count of $18{,}540 \times 10^6$/l, with 93%

neutrophils and 7% lymphocytes, protein 50 g/l, glucose 4.9 mmol/l, lactate dehydrogenase 5510 U/l, and pH 7.0. Peritoneal fluid examination revealed a white cell count of 750×10^6/l, with 35% neutrophils, 3% lymphocytes, and 62% monocytes, protein 7.0 g/l, and glucose 16.1 mmol/l. On day 3 post-incubation, blood culture turned positive with a Gram-negative sea gull-shaped organism. The same organism was also recovered in pleural fluid culture, but not in peritoneal fluid. He responded to cefuroxime and netilmicin and adequate drainage of the empyema and was discharged after 38 days. The bacterial cells were facultatively anaerobic, non-sporulating, Gram-negative, sea gull shaped rods. The organism grew on sheep blood agar as non-hemolytic, gray colonies of 1 mm in diameter after 24 hours of incubation at 37°C in ambient air. For this organism, growth also occurs on MacConkey agar, at 25°C and 42°C, but not at 4°C, 44°C, and 50°C. It can grow in 1%, 2%, but not 3%, 4%, or 5% NaCl. No enhancement of growth is observed in 5% CO_2. The organism is aflagellated, and is non-motile at both 25°C and 37°C. It is oxidase, catalase, urease, and arginine dihydrolase positive, and it reduces nitrate. It does not ferment, oxidize, or assimilate any sugar tested. It is sensitive to ampicillin, cefalothin, cefuroxime, ceftazidime, ceftriaxone, imipenem, azetreonam, erythromycin, clarithromycin, gentamicin, amikacin, ciprofloxacin, levofloxacin, chloramphenicol, tetracycline, co-trimoxazole, polymyxin B, but resistant to vancomycin, clindamycin, metronidazole. 16S ribosomal RNA gene sequencing showed that there are 6.2%, 7.7%, and 8.2% difference between the 16S rRNA gene sequence of the bacterium and those of *Microvirgula aerodenitrificans*, *Vogesella indigofera*, and *Chromobacterium*

species respectively. The G + C content (mean ± SD) is 68.0 ± 2.43%. Based on phylogenetic affiliation, it belongs to the *Neisseriaceae* family of the β-subclass of *Proteobacteria*. The bacterium was named *Laribacter hongkongensis*. Further studies should be performed to ascertain the potential of this bacterium to become an emerging pathogen.

In the second case, a 42-year old Chinese woman was admitted to hospital because of fever, chills, and rigors for 1 week. She had mitral regurgitation as a result of chronic rheumatic heart disease. Examination showed an oral temperature of 38.5°C, a grade 3/6 pansystolic murmur over the cardiac apex radiating to the left axilla (compatible with her mitral regurgitation), and a 3 cm erythematous nodule over the left palm (compatible with a Janeway lesion due to septic embolism). The haemoglobin was 11.5 g/dl, total white cell count 7.5×10^9/l, with neutrophil count 6.0×10^9/l, lymphocyte count 1.0×10^9/l, monocyte count 0.4×10^9/l, and platelet count 243×10^9/l. The serum albumin, globulin, creatinine, urea, bilirubin, alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase were within normal limits. The erythrocyte sedimentation rate was 37 mm/h. A presumptive diagnosis of infective endocarditis was made. Three sets of blood cultures were obtained and empirical intravenous ampicillin and gentamicin were commenced. Both transthoracic and transesophageal echocardiograms did not reveal any vegetations. On day 1 post-incubation, all 3 pairs of blood cultures turned positive with the same Gram-positive coccus. She responded to a total of 4 weeks of ampicillin and 2

weeks of gentamicin. The bacterial cells were facultatively anaerobic, non-sporulating, Gram-positive cocci arranged in chains. The organism grows on sheep blood agar as α-haemolytic, gray colonies of 0.5 - 1 mm in diameter after 24 hours of incubation at 37°C in ambient air. Growth also occurs in 10% or 40% bile and on bile esculin agar, but not in 6% NaCl. No enhancement of growth is observed in 5% CO_2. It is non-groupable with Lancefield groups A, B, C, D, F, or G antisera, and is resistant to optochin and bacitracin. The organism is aflagellated, and is non-motile at both 25°C and 37°C. It is Voges-Proskauer test positive. It produces leucine arylamidase and β-glucosidase, but not catalase, urease, lysine decarboxylase, or ornithine decarboxylase. It hydrolyses esculin and arginine. It utilizes glucose, lactose, salicin, sucrose, pullulan, trehalose, cellobiose, hemicellulase, mannose, maltose, and starch. It is sensitive to penicillin (MIC = 0.064 μg/ml), ceftriaxone, cefepime, clindamycin, erythromycin, ofloxacin, tetracycline, and vancomycin. 16S rRNA gene sequencing showed that there are 3.6%, 3.7%, 4.3%, 4.7% and 5.9% differences between the 16S rRNA gene sequence of the bacterium and those of *Streptococcus gordonii*, *Streptococcus intermedius*, *Streptococcus constellatus*, *Streptococcus sanguis*, and *Streptococcus anginosus* respectively. The G + C content of it (mean ± SD) was 53.0 ± 2.9%. Based on phylogenetic affiliation, it belongs to the mitis or anginosus group of *Streptococcus*. The bacterium was named *Streptococcus sinensis*. Further studies should be performed to ascertain the potential of this bacterium to become an emerging cause of infective endocarditis.

References

1. Yuen KY, Woo PCY, Teng JLL, Leung KW, Wong MKM, Lau SKP. *Laribacter hongkongensis* gen. nov., sp. nov., a novel Gram-negative bacterium isolated from a cirrhotic patient with bacteremia and empyema. *Journal of Clinical Microbiology* 2001; 39: 4227-4232.
2. Woo PCY, Tam DMW, Leung KW, Lau SKP, Teng JLL, Wong MKM, Yuen KY. *Streptococcus sinensis* sp. nov., a novel *Streptococcus* species isolated from a patient with infective endocarditis. *Journal of Clinical Microbiology* 2002; 40: 805-810.

Community acquired pneumonia: local scene

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Community acquired pneumonia (CAP) is a common and important cause of morbidity and mortality in every locality. Although several practice guidelines have been published in the past few years in North America and Europe[1-6], all these "Western" recommendations are based on their own local epidemiological data, resistance patterns and their unique health delivery systems, and they may not be completely applicable to Hong Kong. Thus it is very important to have our own local data and preferably, our own "CAP Guideline".

Local epidemiology

A local prospective study[7] of CAP was done at Prince of Wales Hospital (PWH) more than a decade ago. The etiologic diagnosis of pneumonia was made in 41% of the cases. Tuberculosis and *S. pneumoniae* were the most common causes of CAP in this study. CAP caused by atypical pathogens (*Chlamydia* or *Mycoplasma pneumoniae*) constituted 8.9% of this study group. Another study focusing on viral, mycoplasmal and chlamydial lower respiratory tract infection done at the same hospital[8] showed that mycoplasma pneumonia was mainly found in young patients. A more recent prospective study of CAP done at Alice Ho Miu Ling Nethersole Hospital showed that *H. influenzae* was the most common cause of CAP. *Mycoplasma* pneumonia was identified in 8.7% of cases[9]. The laboratory data from Queen Elizabeth Hospital in 2000 (presented at Joint Scientific Meeting of Infectious Disease and Ambulatory Medicine, Oct 2001) also showed that *H. influenzae* was the most common cause of CAP. Mycoplasma pneumonia was definitely not uncommon.

Local resistance pattern

DRSP

The isolation of drug resistant *Streptococcus pneumoniae* (DRSP) in Hong Kong was first reported in 1990[10]. The percentage of moderate to highly resistant pneumococcus has risen to 70% in 1998[11]. Reports from PWH[12,13] showed that the percentage of reduced penicillin susceptibility rose rapidly from 10% in 1993 to 50% in 1997, and reached 63% in years 1999 - 2000. The prevalence of erythromycin resistance in pneumococci has also risen from 22% in 1993 to 71% in year 1999 – 2000 while the mean rate of resistance to tetracycline and co-trimoxazole (1993 - 7) were 78% and 55% respectively. In Princess Margaret Hospital, the resistance rates to penicillin, erythromycin and levofloxacin were 2 - 14%, 58 - 65% and 7 - 9% respectively (Data of 1998 - 2000).

Relationship between the phenotypic and genotypic characteristics of 105 penicillin-intermediate or resistant *S. pneumoniae* isolates at PWH and Pamela Youde Nethersole Eastern Hospital is analysed[14]. 74% of the strains belonged to serotypes 23F, 19F and 14. The 23F isolates may be variants of the Spanish 23F clone. Serotype 6B accounted for 19% of the isolates with reduced penicillin susceptibility, being indistinguishable from the Spanish 6F clone. Hence the rapid emergence of drug resistant *S. pneumoniae* in Hong Kong was likely due to rapid dissemination of several successful clones.

LRSP

The emergence of fluoroquinolone resistant *S. pneumoniae* (LRSP) in Hong Kong was first reported in 1999[11]. Resistance to ciprofloxacin, levofloxacin and trovafloxacin was found in 12.1, 5.5 and 2.2% of the strains respectively. Further case study[15] with multivariate analysis showed that presence of COPD, residence in a nursing home, nosocomial origin of the bacteria and exposure to quinolones were independently associated with LRSP colonisation or

infection. A distinct group of patients with COPD is the reservoir of LRSP. Widespread usage of ciprofloxacin and ofloxacin, both of which have only limited anti-pneumococcal activities, and nosocomial spread of pneumococcus, may be the potential explanation.

According to one recent report, the prevalence of fluoroquinolone non-susceptibility has risen to 13% in 2000 in Hong Kong[16]. All these non-susceptible isolates were mainly derived from elderly and COPD patients. These isolates were all non-susceptible to penicillin, cefotaxime and erythromycin. The newer generations of fluoroquinolones were not the solution to this problem because almost all levofloxacin non-susceptible isolates were also intermediate or resistant to gatifloxacin and moxifloxacin. A potential solution to LRSP comes from the recent report of activity of linezolid against levofloxacin resistant *S. pneumoniae*[17]. However, available clinical data on the role of linezolid in drug resistant *S. pneumoniae* are still limited.

Erythromycin resistance in *S. pneumoniae*

Erythromycin resistance in *S. pneumoniae* is also very common in Hong Kong[18]. Erythromycin resistance among penicillin-susceptible *S. pneumoniae* was 38%, and among penicillin-intermediate and resistant isolates was 92%. 27% of the isolates showed the MLS_B phenotype, and the majority carried the *ermB* gene. 73% displayed the M phenotype, and all possessed the *emf* gene. Cases of pneumococcal infection caused by strains demonstrating high-level cefotaxime resistance (MIC, 4 mcg/ml) had also been reported in Hong Kong[19]. Both patients died despite treatment with third generation cephalosporin.

Treatment recommendation

There is still no widely accepted local guideline for the management of CAP. In general, the “Western Guidelines” are more similar than different.

Treatment recommendation depends on the patient stratification: place of therapy; the presence of cardiopulmonary disease and modifying factors (risk factors for DRSP, Pseudomonas aeruginosa and enteric Gram negative infections). For outpatient treatment, macrolide or doxycycline is recommended for those without modifying factors while quinolones or beta-lactam plus macrolide are recommended for those with modifying factors.

The availability of “respiratory” quinolones (e.g. levofloxacin, gatifloxacin, moxifloxacin, etc.) have revolutionised the treatment strategy. However, quinolone is not recommended to be the first line treatment for CAP in Hong Kong because tuberculosis is a common cause of pneumonia in Hong Kong. The use of quinolone may cause potential problems in both the diagnosis of TB and the development of resistance. CDC recommends that fluoroquinolones should be limited to those who either are allergic to the first line agents; who have failed first line therapy; or who have been documented to have highly drug resistant pneumococcal infection (MIC $\geq$ 4 mcg/ml)[3]. The option of doxycycline is also unlikely to be useful in Hong Kong because the resistance rate of pneumococcus to tetracycline is more than 70%[12].

Concerning the inpatient treatment, either the combination of beta-lactam plus macrolide or monotherapy with fluoroquinolone should be considered. The addition of macrolide to beta-lactam containing regimen was optional in previous CAP guidelines. However, Gleason et al had shown nicely that the combination therapy could lead to a lower 30-day mortality[20]. Hence all the guidelines published after 1999 have added macrolide.

In the ATS and Canadian guidelines, treatment for patients requiring ICU care is further stratified on the basis of whether risk for *P. aeruginosa* is present or not[1,2]. In the absence of pseudomonal risk factors, therapy should be with a beta-lactam active against DRSP plus a quinolone or macrolide. Cefotaxime and ceftriaxone have superior activity against resistant pneumococci in

comparison with ampicillin-sulbactam or with cefuroxime. “Selected” beta-lactams with activity against both *DRSP* and *P. aeruginosa* (e.g. cefepime, carbapenams, piperacillin / tazobactam) should be chosen for those with risk of *P. aeruginosa* infection. The role of fluoroquinolone monotherapy in severe CAP is currently uncertain. The published clinical trials have generally involved few patients admitted to ICU, and the proper dosing and efficacy of the new quinolones for severe CAP is unknown[1].

IMPACT guideline[21] is the only local formal guideline that gives official recommendation for antimicrobial therapy of CAP in Hong Kong. IMPACT guidelines recommend Augmentin / Unasyn +/- a macrolide or amoxicillin + a newer macrolide for outpatient therapy; Augmentin / Unasyn / cefotaxime / ceftriaxone +/- a macrolide for patients hospitalised in general ward; Tazocin / cefotaxime / ceftriaxone / cefepime plus a macrolide for patients hospitalised in ICU. According to this guideline, macrolide / azalide, tetracycline or co-trimoxazole should not be used alone for empiric treatment of CAP. Overall, it looks more similar to the European guideline[4] than the American or Canadian guidelines.

Besides bronchiectasis, there is no further information about patient stratification in this IMPACT guideline. The importance of performing patient stratification has been fully elaborated in various international guidelines. Essentially, the place of therapy (outpatient, inpatient or ICU care) is a reflection of severity of illness. The most likely pathogens for each patient group are different. Not all patients in areas with high rates of DRSP are likely to be infected with these organisms and clinical risk factors have to be defined. Only those few patients with specific modifying factors will suffer from CAP caused by *enteric Gram-negative organisms* or *Pseudomonas aeruginosa.* Hence, it will be impossible for any single one regimen to be optimal for every patient group. ATS[1] and Canadian[2] guidelines give the best account in this aspect.

The three American[1,3,5] and Canadian[2] guidelines emphasised the necessity of empiric coverage of atypical pathogens in every patient with CAP. This recommendation is based on the high frequency of these pathogens, often in the form of mixed infection with a bacterial pathogen. Local studies[7,9] also have consistently showed that around 8% of CAP was caused by atypical pathogens. The mean age of patients with mycoplasma pneumonia was 33 years old[9]. Although it may be unnecessary to cover the atypical pathogens empirically for every patient with CAP[6], atypical pathogens should be considered in young patients and during the epidemic of mycoplasma infection.

Can macrolide monotherapy be used in patients with CAP? In view of the emerging high rate of in vitro resistance of *S. pneumoniae* to macrolides, many people have objection to macrolide monotherapy for CAP in Hong Kong. Whether in vitro resistance will translate into clinical treatment failure is controversial. This is because newer macrolides (clarithromycin, azithromycin) have very unique pharmacology. Once absorbed, macrolides and azalides are avidly taken up by leucocytes that are chemotactically attracted to the infection site. These cells not only release drug at the site of infection but also, after phagocytosis of the pathogen, expose it to high intracellular drug concentration. Published evidence for clinical failure of macrolides in the treatment of pneumococcal pneumonia remains limited and controversial. British CAP guideline takes an open attitude towards this controversial area[6]. The combination of amoxicillin and a newer macrolide should provide adequate coverage of both typical and atypical pathogens for CAP in Hong Kong.

The addition of macrolide to beta-lactam containing regimen for patients hospitalised in general ward with CAP still remains optional in IMPACT guideline. Since superior clinical outcomes have been demonstrated unequivocally with combination antibiotic therapy[20], most authoritative

guidelines (except European guideline) have recommended combination therapy (beta-lactam with macrolide) for hospitalised patients (in general ward) . It should be noticed that European guideline was published before Gleason's landmark paper. Unless there is very strong local data against this international consensus, the proposal of beta-lactam monotherapy for inpatient seems unjustified. For those with beta-lactam allergy, a respiratory fluoroquinolone monotherapy should be recommended as an alternative.

Pneumococcal Vaccination

Resistant strains of pneumococcus are confined to a limited number of serogroups notably 23F, 19F, 14, 6F which account for more than 90% of DRSP. These resistant strains are fully covered in the 23-valent polysaccharide vaccine which has good efficacy in adults and children above 2 years old. Vaccination of at risk patients is not yet fully utilised in Hong Kong. The cost effectiveness of pneumococcal vaccination should be further explored in the future.

Reference

1. Guidelines for the management of adults with community acquired pneumonia. Diagnosis, assessment of severity, antimicrobial therapy, and prevention. Am J Respir Crit Care Med 2001; 163:1730-1754
2. Canadian guidelines for the initial management of community acquired pneumonia: An evidence-based update by the Canadian Infectious Disease Society and the Canadian Thoracic Society. CID 2000;31:383-421
3. Management of community acquired pneumonia in the Era of pneumococcal resistance. Arch Intern Med 2000;160:1399-1408
4. Guidelines for management of adult community acquired lower respiratory tract infections. Eur Respir J 1998;11:986-991

5. Practice guidelines for the management of community acquired pneumonia in adults. CID. 2000;31:347-82
6. BTS Guidelines for the management of community acquired pneumonia. *Thorax 2001;56(suppl IV)*
7. Chan HS et al. A prospective study of community acquired pneumonia in Hong Kong. Chest 1992;101:442-46
8. Bassett DCJ et al. Viral, mycoplasmal and chlamydial lower respiratory tract infections in Hong Kong: cost and diagnostic value of serology. Br J Biomed Sci 1994;51:9-13
9. Chan HS et al. A prospective study of community –acquired pneumonia in Hong Kong. Free paper presentation in Annual Respiratory Scientific Meeting March 2002
10. Yuen KY et al. Multi-resistant Streptococcus pneumoniae in Hong Kong. J Antimicrob Chemother 1990;25:721-3
11. Ho PL et al. Emergence of fluoroquinolone resistance among multiple resistant strains of Streptococcus pneumoniae in Hong Kong. Antimicrob Agents Chemother 1999;43:1310-3
12. Ip M et al. Patterns of antibiotic resistance, serotype distribution, and patient demographics of Streptococcus pneumoniae in Hong Kong. Chemotherapy 2001;47:110-6
13. Ip M et al. Penicillin and Macrolide resistance in Streptococcus Pneumoniae in Hong Kong-What actually happened? Free paper presentation in Fifth Annual Scientific meeting of The Hong Kong Society for Infectious Diseases
14. Ip M et al. Evidence of clonal dissemination of multidrug-resistant Streptococcus pneumoniae in Hong Kong. J Clin Microbiol Sept 1999,p2834-9
15. Ho PL et al. Risk factors for acquistion of levofloxacin-resistant Streptococcus pneumoniae : A case control study. CID 2001;32:701-7

16. Ho PL et al. Increasing resistance of Streptococcus pneumoniae to fluoroquinolones: results of a Hong Kong multicentre study in 2000. J Antimicrob Chemother 2001;48:659-665
17. Ho PL et. al. Activity of linezolid against levofloxacin-resistant Streptococcus pneumoniae, methicillin-resistant Staphylococcus aureus and vancomycin-resistant enterococci in Hong Kong. J Antimicrob Chemother 2001;48:590-2
18. Ip M et al. Macrolide resistance in Streptococcus pneumoniae in Hong Kong. Antimicob Agents Chemother 2001;45:1578-80
19. Wong SSY et al. Emergence of streptococcus pneumoniae with high level resistance to cefotaxime in Hong Kong . HKMJ 1999;5:406-9
20. Gleason PP et al. Associations between initial antimicrobial therapy and medical outcomes for hospitalized elderly patients with pneumonia. Arch Intern Med 1999;159:2562-2572
21. Reducing bacterial resistance with IMPACT-Interhospital Multi-disciplinary Programme on Antimicrobial Chemotherapy. 2nd ed

Molecular biology of prion disease
(A report from the 10th International Congress on Infectious Diseases, 11 – 14 March 2002, Singapore)

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Dr. C. Weissmann (MRC Prion Unit, Department of Neurodegenerative Disease, Institute of Neurology, London) gave a plenary lecture titled "Molecular Biology on Prion Disease" on the 1st day of the Congress. His lecture helps to clarify some of the concepts on the pathophysiology of the disease, and provides insights on our understanding of this increasingly important subject. Some of the key topics discussed in his lecture will be presented in this report.

Prion diseases are neurodegenerative diseases that have long incubation periods and progress inexorably once clinical symptoms appear. First coined by Dr. Stanley Prusiner in 1982, "prion" is defined as small infectious pathogen containing protein but apparently lacking nucleic acids [1]. They are believed to be the cause of a number of conditions affecting human and animals, collectively known as the "transmissible spongiform encephalopathies", which comprise diseases such as scrapie in sheep, bovine spongiform encephalopathy (BSE) in cattle and Creutzfeldt-Jakob disease (CJD) in man [2]. Recently, over 100 cases of variant CJD (vCJD) have been reported in United Kingdom and elsewhere, and are attributed to consumption of BSE-infected cattle products [3].

The transmissible agent, the prion, is devoid of nucleic acid and consists mainly or entirely of PrP, a modified form of the normal host protein PrPC. PrPC is a glycoprotein found in brain tissue of normal animals [4]. The normal function of PrPC is not known; it can be detected attached to the plasma membrane of neurons [5], may be concentrated at synaptic membranes [6], and is anchored to the cell surface by a phosphatidylinositol glycolipid, involving modification of both the amino and carboxy terminals of the protein [7]. Surface PrPC is normally degraded after endocytosis in acidic vesicles, and some protein may be recycled to cell surface [8], or exist in secreted forms [9].

Scrapie prions have been used as model for prion diseases. The prion protein isolated from the brains of scrapie-infected animals, known as PrPSc, is a conformational isomer of PrPC. In contrast to PrPC, which exists primarily in an α helical conformation, PrPSc is β helical and appears to result from a yet undefined conformational alteration in PrPC [10]. The resistance of PrPSc to digestion with proteases and its tendency to polymerize into scrapie-associated fibrils or rods further differentiates it from PrPC [11]. PrPSc tends to

accumulate within cells and does not normally appear on the cell surface; it is predominantly found in cytoplasmic vacuoles and secondary lysosomes [12]. The hydrophobic nature of the protein, its tendency for aggregation, as well as the β sheet conformation may all play a role in neurotoxicity [13].

Studies with mice either devoid of PrPC or with abnormal isoforms indicate that host PrPC must be present for development of prion disease. As an example, PrP knockout (Prnpo/o) mice are resistant to scrapie [14]. Prion diseases appear to result from accumulation of abnormal isoforms of the PrP, which is dependent upon conversion of normal PrPC to PrPSc. It has been hypothesized that the abnormal protein itself might bring about this conversion [3]; the initiating PrPSc molecule might be derived from an exogenous source, as in sporadic and iatrogenic diseases. On the other hand, mutations to the prion protein gene (PRNP gene) might account for the development of familial cases, as these mutations could destabilize PrPC, leading to spontaneous conversion to PrPSc [15]. As predicted by this "protein-only" hypothesis, the introduction of PrP transgenes into PrP knockout mice restores susceptibility to scrapie.

Prions are unusually resistant to inactivation by heat, chemical agents or radiation. Iatrogenic transmission, by introduction of contaminated biological preparations has caused more than 100 cases of CJD. In particular, stainless steel instruments may retain infectivity even after treatment with 10% formaldehyde [16]. The presenter's group has established experimental models studying transmission of disease through brain electrodes recently. It was found that steel, gold or plastic surfaces are able to bind prions tenaciously, even after 5 mins exposure to infected brain homogenates, and can transmit them to indicator mice when inserted intracerebrally for as little as 30mins [17]. No protein can be eluted from the infectious wires; it was also found that metal-surface-bound prions could infect cultured cells only when there was direct contact between the cells and the wire, suggesting that metal-bound prions can initiate the conversion process at the surface of PrP-bearing cells. These findings provide important insights into the pathogenesis of the disease.

There is currently no therapy for prion diseases. Since abrogation of PrP synthesis can prevent pathogenesis and prion propagation and does not seem to be deleterious, drugs specifically inhibiting PrP synthesis, its transport to cell surface or substantially accelerating its degradation are potential treatment options. For instance, it was recently discovered by the presenter's group that treatment of scrapie-infected neuroblastoma cells with a PrP antibody cleared the infection in 3 days [18], suggesting that there is rapid turnover of PrPSc leading to disappearance if not replenished by conversion of PrPC. Therapeutic options in the future may thus include passive or active immunizations.

References

1. Prusiner SB. Science 1982; 216:136
2. Prusiner SB. N Engl J Med 2001; 344: 1516
3. Collinge J. Lancet 1999; 354:317
4. Harris DA. Clin Microbiol Rev 1999; 12:429
5. Narwa R, Harris DA. Biochemistry 1999; 38:8770
6. Herms J, Tings T, Gall S et al. J Neurosci 1999; 19:8866
7. Harris DA, Huber MT, van Dijken P et al. Biochemistry 1993; 32:1009
8. Shyng SL, Huber MT, Harris DA. J Biol Chem 1993; 268:15922
9. Hay B, Prusiner SB, Lingappa VR. Biochemistry 1987; 26:8110
10. Downing DT, Lazo ND. Biochem J 1999; 343 Pt 2:453
11. Prusiner SB, McKinley MP, Bowman KA et al. Cells 1983; 35:349
12. Borchelt DR, Taraboulos A, Prusiner SB. J Biol Chem 1992; 267:16188
13. Jobling MF, Stewart LR, White AR et al. J Neurochem 1999; 73:557
14. Bueler H, Aguzzi A, Sailer A et al. Cell 1993; 73:1339
15. Cohen FE, Prusiner SB, Ann Rev Biochem 1998; 67:793
16. Zobeley E, Flechsig E, Cozzio A et al. Mol Med 1999; 5:240
17. Flechsig E, Hegyi I, Enari M et al. Mol Med 2001; 7(10): 679
18. Enari M, Flechsig E, Weissmann C. Proc Natl Acad Sci USA 2001 31; 98(16): 9295

Emergence of Nipah Virus: How it happened? Will we have another epidemic?

(A report from 10th International Congress on Infectious Diseases, 11 – 14 March 2002, Singapore)

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The lecture was given by Dr. K. B. Chua of University of Malaysia, Department of Medical Microbiology, Kuala Lumpur, Malaysia. In September 1998, a new paramyxovirus named Nipah virus was found and causing fatal disease in domestic pigs and human in Malaysia. It is a RNA virus which was isolated from the cerebrospinal fluid of a number of patients with viral encephalitis. The virus was transmitted through the respiratory droplets of the pigs to human who handled the animals. After the outbreak, the group of Dr. Chua tried to find out the reservoir and the source of the virus.

Dr. Chua and his group members visited the forests and tried to take samples from the fruit bats in there. They took the urine samples of the bats and cultured them for the Nipah virus. Other than this, they also took swabs from the fruits which were bitten by the bats hoping that the virus was also excreted from the saliva. Both of these samples yielded the Nipah virus. Although not all the samples were positive, they thought that the fruit bats were the natural reservoir of the virus.

In the past twenty years, the natural habitats of the fruit bats in Asia were disturbed and destroyed by massive deforestation for timber, pulpwood and industrial plantation. The bats had to find a new habitat. Also the El Nino Southern Oscillation event which caused severe drought had led to the migration of the bats to the cultivated fruit orchards in 1997/1998. The locations of the pig farms in the orchard made the transmission of the virus possible from its reservoir. Dr. Chua had concluded that as long as the reservoir is there, when El Nino comes, the migration of the bats could potentially cause outbreak in Southeast Asian countries. So we have to alert for this disease and infection in the coming years.

A young lady with weight loss, multiple skin lesions and convulsion

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Case Report

A previously well 29-year-old married housewife was admitted in October 2000 because of repeated convulsions and fever. She had been married for 3 years, had enjoyed good past health, and had no reported risk factors for HIV infection. Prior to this admission, she had night sweats, productive cough and significant weight loss for few months. She was admitted to another hospital in August 2000 due to fever and haemoptysis. Chest radiograph and computed tomography of thorax at that time showed consolidation of the right lower lobe. She was diagnosed to have pneumonia and treated with a course of co-amoxiclav and clarithromycin. Sputum cultures were negative for pyogenic organisms and *Mycobacterium sp*. Despite some initial response to treatment, she continued to have persistent chest symptoms, and she also noticed the gradual appearance of multiple painless skin nodules over her body.

In the week before her admission to our hospital, she began to have episodes of fever. She also noticed a few episodes of transient limb weakness and involuntary limb twitching. On the day of admission, she was witnessed to have repeated episodes of generalized tonic-clonic convulsions. On examination, she was noted to be febrile (39.5°C) and cachexic (BW = 46 kg), with a scalp ulcer over the vertex of skull, and another skin ulcer over the right arm. There was also an indurated area over the abdominal wall. The rest of the examination, including neurological examination, was normal. A chest radiograph showed a right lower zone infiltrate. Blood tests showed mild normochromic normocytic anaemia (Hb = 11.3) with normal WCC (8.4, 87% neutrophils, 5% lymphocytes). Renal and liver function tests were normal, apart from hypoalbuminaemia (Alb = 29); spot glucose, bone profile, thyroid function tests were normal, and immune markers (ANA, ANCA, ACL) were negative. ESR and CRP were markedly elevated. Routine blood and sputum cultures were negative. Computed tomography of the brain revealed multiple contrast-enhancing intra-cranial lesions with vasogenic oedema, which were T1 iso-intense and T2 hyper-intense on magnetic resonance imaging. As the lesions were thought to represent multiple brain abscesses, intravenous cefotaxime and metronidazole were commenced, and a neurosurgical opinion was sought. The low-grade fever persisted despite the initiation of antibiotics; the neurosurgical team suggested conservative management instead of drainage, as the brain lesions were relatively small in size. Skin biopsy of the scalp lesion was performed, with histology showing the presence of acute inflammation with occasional granulomas; all routine bacteriologic and fungal stains were negative. Pus was aspirated from the abdominal wall lesion and sent for staining and culture.

Subsequent investigations revealed that the patient was HIV positive, with a low CD4 count (26/μL). The pus aspirated from the abdominal wall lesion yielded a slow-growing organism, which was a Gram +ve, aerobic branching rod, weakly acid-fast, and later identified as *Nocardia asteroides*. It thus became clear that the patient was suffering from disseminated nocardiosis, as a presenting illness of her HIV infection. She was treated with amikacin and septrin, and responded with rapid resolution of fever and symptoms. Repeated imaging also showed resolution of intracranial lesions (now presumed to be brain abscesses caused by *N. asteroides*), and all her skin lesions also healed completely. She was started on HAART and followed up at Queen Elizabeth Hospital. One year after her initial presentation, she remains well with no signs of relapse of her disease.

Discussion

Nocardia belongs to a genus of aerobic actinomycetes capable of causing localized or systemic suppurative disease in humans and animals [1]. Initially isolated from cattle by Edmond Nocard in 1888, the first human case was reported by Eppinger in 1890. Although frequently reported to cause disease in immunocompromised hosts, it is also associated with disease in "normal" hosts in up to 36% of the cases [2-5]. The actinomycetes is actually an informal group of aerobic and anaerobic bacteria in the order of Actinomycetales, grouped together because they share similar morphology; other genera of the group with medical importance include *Rhodococcus*, *Mycobacterium* and *Corynebacterium* [6]. Of the different subgroups of *Nocardia*, *N. asteroides* is the organism most frequently isolated, accounting for up to 80 - 90% of cases; other subgroups include *N. brasiliensis* (causative agent of mycetoma), *N. farcinica* (antibiotic resistant and virulent, often causing disseminated disease), *N. nova, N. otitidiscaviarium*, and *N. transvalensis* [2]. Being environmental saprophytes, they are found worldwide in soil, decaying vegetable matters and aquatic environments. They can thus easily contaminate food sources, be carried airborne on dust particles (subsequently inhaled causing pulmonary infections), and be inoculated into skin (causing cutaneous infections) [2]. In the United States a total of 500-1000 cases are reported annually; the exact local prevalence is unknown, although a prevalence of 2.8% among SLE patients has been quoted in a case series [7].

Cell mediated immunity (CMI), as opposed to humoral immunity, is believed to be an important facet of the host defense mechanism against nocardiosis. In early infection, neutrophils and macrophages were found to inhibit but not kill the bacteria. Specific virulence factors of the bacteria include anti-phagocytic properties, the ability to inhibit phagosome-lysosome fusion, and the presence of toxic cell wall glycolipids, which inhibit intracellular killing. In addition, *Nocardia sp.* is capable of existing in cell wall deficient forms ("L-form") in adverse conditions, which may account for life-long persistence and late relapses within the host [8,9]. Having considered the pathogenetic mechanisms underlying human infection, it is not surprising that up to 65% of affected cases were found to have associated immune dysfunction, particular defective CMI, in a

case series involving 1050 patients [1]. Among the host risk factors identified were prolonged steroid use, in particular in autoimmune diseases (e.g. SLE, asthma); chronic lung diseases, such as COAD and bronchiectasis; haematological malignancies, other neoplasms and bone marrow transplant recipients; HIV infection; diabetes mellitus, alcoholism, tuberculosis, and alveolar proteinosis. An increased male to female ratio is also noted in some case series [3,4].

The clinical manifestations of the disease can be subdivided into the following categories: pulmonary disease, extra-pulmonary or disseminated disease, central nervous system (CNS) disease and cutaneous disease. Nocardiosis in patients with HIV infection will be considered as a separate entity in the following discussion.

Pulmonary nocardiosis: This is the predominant finding in > 40% of reported cases [2]. It can occur as an acute, subacute, or chronic process, often accompanied by non-specific symptoms and constitutional upset. A wide variety of radiographic findings have been reported; these include pulmonary nodules (single or multiple), lung masses with or without cavitation, reticulonodular infiltrates, interstitial infiltrates, lobar consolidation, subpleural plagues, and pleural effusions [10]. Due to the non-specific presentation, misdiagnosis of pulmonary nocardiosis as lung abscess, tuberculosis, fungal disease and malignancy may occur [2]. Complications can occur as a result of local or systemic dissemination from a pulmonary focus; local complications include development of empyema, mediastinitis, pericarditis, and even superior vena cava syndrome. Unlike in localized pulmonary disease, where treatment with antibiotics alone usually results in favourable outcome, surgical drainage is often required when complication occurs. As an example, in a case series involving patients with pericardial nocardiosis, the outcome was uniformly fatal without surgical drainage [11].

Extra-pulmonary nocardiosis: This is usually the result of dissemination from a pulmonary or cutaneous focus, although the original source may not be apparent. Haematogenous spread to bone, retina, heart, joints and kidneys have been commonly reported, though theoretically any site is possible. “Disseminated nocardiosis” is defined as involvement of ≥ 2 non-contiguous sites and signifies a poor prognosis; mortality rates of up to 44% in immunocompetent hosts and >85% in immunocompromised hosts have been reported [12].

CNS nocardiosis: Various studies have suggested that *Nocardia sp.* may have a specific tropism for neural tissue. *Nocardia sp.* has been implicated in neurodegenerative disorders in murine models, including a variant form of Parkinsonism [13]. CNS involvement by *Nocardia* is common; overall, it has been reported in 20% of all cases, and up to 44% of disseminated cases in a large series [2]. Cases with “isolated” CNS involvement probably result from dissemination from occult or transient pulmonary foci of infection. CNS nocardiosis frequently results in the formation of parenchymal abscess, often loculated in nature, although rarely it can also cause meningitis. These lesions can

remain indolent for years, resulting in misdiagnosis as brain tumours; CNS nocardiosis can also present with fever, meningismus, or seizures [14]. Open drainage of nocardial brain abscess is often recommended, except for small lesions (< 2 cm) in immunocompetent patients [15].

Cutaneous nocardiosis: Primary cutaneous nocardiosis often results from direct inoculation of *Nocardia sp.* from trauma or insect bites [16]. Another form, the so-called "sporotrichoid nocardiosis", occurs when there is extensive regional lymphatic involvement [17]. Rarely, in up to 2% of cases, skin lesions may be the initial presentation in disseminated disease [18]. *Nocardia sp.* in particular *N. brasiliensis,* can also be the cause of mycetoma, a chronic cutaneous infection characterized by formation of sinus tracts exuding granules, often with underlying muscle and bone involvement.

The overall prognosis depends on the site and extent of disease, as well as host factors. Cure rates of up to 100% can be expected in isolated soft tissue or skin involvement. However, while a cure rate of up to 90% has been reported in pleuropulmonary disease, the cure rate drops sharply to 60% when there is disseminated disease [19]. In the case of CNS involvement with brain abscess formation, a cure rate of 50% has been reported, although a worse prognosis is to be expected if there are multiple abscesses, or if the host is immunocompromised [14].

Nocardiosis in HIV: HIV infection is now the second leading predisposing factor for nocardiosis after organ transplantation according to a large series [2]. Although overall an uncommon opportunistic infection, the frequency of disease among AIDS patient in the United States has increased from 0.3% in 1985 to 1.8% in 1989 [20]. Although nocardiosis is not included in the 1993 CDC case definition, many authorities would regard nocardiosis as an AIDS-indicator condition [21]. According to a classic case series [22,23], HIV infected patients with nocardiosis tend to have advanced disease, with low CD4 count (mean = 104). Many would have co-existing or antecedent AIDS-defining conditions, and co-pathogens such as *M. tuberculosis* are common. Pulmonary nocardiosis is still the most common form of presentation; chest radiographs frequently show alveolar infiltrates, although a peculiar pattern with upper lobe cavitary change is also reported (c.f. Rhodococcus equi). Up to 50% of HIV infected patients with nocardiosis are found to be intravenous drug users; a disseminated pattern of involvement, with formation of mediastinal, retroperitoneal and brain abscesses has been reported [24]. Relapse is common and prolonged prophylaxis after treatment has been the standard recommendation. An overall attributable mortality of up to 70% has been reported; poor outcome is associated with delayed diagnosis, extensive disease, as well as early discontinuation of treatment [22].

Diagnosis of nocardiosis relies on isolation and identification of the organism from clinical specimens. Delay in diagnosis is common; a mean period of up to 12 months has been reported. In addition, use of invasive procedures is often needed; for instance, up to

44% of pulmonary infections require use of invasive procedures to obtain adequate specimens for diagnosis [25]. Recovery of the organism from the laboratory can be similarly difficult; if *Nocardia sp.* is suspected, decontamination of sputum specimens with sodium hydroxide, N-acetyl-L-cysteine, and benzalkonium chloride should be avoided [6]. Although most media would support the growth of *Nocardia sp.*, the use of certain selective media such as BCYE and modified Thayer-Martin may be preferred for specimens from non-sterile sites [6], to suppress overgrowth of other organisms. *Nocardia sp.* are slow growing, requiring prolonged incubation in laboratory (up to 4 weeks for blood cultures) [4]; hence laboratory personnel should always be alerted if nocardiosis is suspected, so as to avoid discarding specimens prematurely. Clinical specimens suspected to contain *Nocardia sp.* can also be subjected to acid-fast staining by the modified Kinyoun procedure (acid alcohol substituted by 1% sulphuric acid); a presumptive diagnosis can be made if partially acid-fast filamentous branching rods are seen. Susceptibility testing of Nocardia sp. in the laboratory remains a controversial issue, as no specific NCCLS breakpoints have been established to guide the interpretation of the MICs of various antimicrobial agents. Some authorities recommend that susceptibility testing should be reserved in cases with deep seated or disseminated infections, when there is lack of response to initial treatment or relapsed disease, or when use of second-line agents is contemplated.

No randomised trial has been conducted to define the optimal treatment regime for nocardiosis. Whenever possible, one should reduce the dosage of immunosuppressive agents. Sulphonamides have been considered the standard of therapy for over 50 years, based on retrospective reviews showing a trend towards increased survival [12]. In particular, trimethoprim-sulphamethozaxole (i.e. septrin) is regarded by many as the drug of choice, due to potential synergism between the compounds, high serum concentration achieved, as well as good CNS penetration [27]. If a sulphonamide is used alone, a dose of 6 to 12 gm/day, with an initial dose of 4 gm is recommended; for trimethoprim-sulphamethoxazole, a dose of 2.5 to 10 mg/kg for the trimethoprim component, together with 12.5mg/kg for the sulphamethoxazole component is recommended (i.e. ~ 4 to 6 tablets of septrin 960 mg/day) [10]. If septrin cannot be tolerated, or in case of treatment failure, alternative agents that have been shown to have activity against *Nocardia sp.* include amikacin, imipenem, third generation cephalosporins and minocycline [18]. Most authorities recommend a prolonged treatment course; the maximum tolerated dose should be given for the initial 6 weeks followed by reduced dose for 6 to 12 months [28]. All immunocompromised patients and those with CNS involvement should be treated for at least 1 year [22], while a shorter course may be possible for immunocompetent patients with localized disease. The combination of a sulphonamide with amikacin for the initial 2 weeks should be considered in those critically ill or immunocompromised patients; amikacin can also be combined with ampicillin-sulbactam or imipenem in cases of sulphonamide intolerance. Many authorities would recommend indefinite suppressive therapy in patients whose immunocompromised states cannot be reversed e.g. HIV patients [29]. Surgical drainage should be considered for sequestered abscesses,

mediastinal complications, and for selected cases of brain abscesses. In all cases, the importance of close monitoring for symptoms and signs of relapse cannot be overstated.

In summary, nocardiosis should be considered in compromised patients including those with HIV infection. Clinicians should always maintain a high index of suspicion for this emerging infection. Early diagnosis and treatment for sufficient duration is necessary to ensure an optimal outcome. Close liaison with the microbiology laboratory is vital for timely diagnosis of *Nocardia* infection in all cases.

References

1. Beaman BL et al. J Infect Dis 1976; 134:286
2. Beaman BL et al. Clin Microbiol Rev 1994; 7:213
3. Palmer DL et al. Medicine 1974; 53:391
4. Kontoyiannis DP et al. Medicine 1998; 77:255
5. Curry WA Arch Intern Med 1980; 140:818
6. McNeil MM et al. Clin Microbiol Rev 1994; 7:357
7. Mok CC et al. Semin Arthritis Rheum 1997; 26(4): 675
8. Beaman BL et al. Infect Immun 1978; 20:290
9. Beaman BL et al. Infect Immun 1985; 47:135
10. Lerner PL Clin Infect Dis 1996; 22:891
11. Kessler R et al. Ann Thorac Surg 1991; 52:861
12. Wilson JP et al. Medicine 1989; 68:38
13. Beaman BL Gene 1992; 115:213
14. Mamelak AN et al. Neurosurgery 1994; 35:622
15. Fleetwood et al. Surg Neurol 2000; 53:605
16. Paredes BE et al. Dermatology 1999; 198:159
17. Tsuboi R et al. Arch Derm 1986; 122:1183
18. Threlkeld SC et al. Curr Infect Dis 1997; 17:1
19. Smego RA et al. Arch Int Med 1983; 143:711
20. Holtz et al. Ann Intern Med 1985; 102:203
21. Bartlett JHH et al. Medical care of patients with HIV infection 2001
22. Uttamchandani RB et al. Clin Infect Dis 1994; 18:348
23. Marquez-Diaz et al. AIDS Patient Care and STDs 1998; 12(11): 825
24. Kim et al. Rev Infect Dis 1991; 13:624
25. Georghiou PR et al. Med J Aust 1992; 156:692
26. Mandell et al. Principles and Practice of Infectious Diseases 2000
27. Beaumont R et al. Med J Aust 1970; 2:1123
28. Wallace RJ et al. Rev Infect Dis 1982; 4:315
29. Simpson GL et al. Rev Infect Dis 1981; 3:492