Bulletin of the Hong Kong Society for Infectious Diseases

Vol. 12, no. 2 June 2008

Contents

Streptococcus milleri group infection

W. S. Leung, Department of Medicine, Kwong Wah Hospital

The nomenclature, identification and classification of "*Streptococcus milleri*" has been confusing. It was named by Guthof in 1956 in reference to non-hemolytic streptococcal species isolated from oral infection [1]. The species name was chosen to honor the microbiologist W. D. Miller. Later, Colman and Williams proposed that small beta-hemolytic streptococci along with other non-hemolytic streptococci group F, C or G streptococci collectively referred to as the "*Streptococcus milleri group*" [2]. Coykendall proposed the unification of these streptococci into a single species *Streptococcus anginosus* which was the oldest approved name for these bacteria and therefore had precedence over the name "*Streptococcus milleri*" [3]. More recently, Whiley et al performed DNA relatedness studies on strains classified as *Streptococcus anginosus*, and observed that three DNA homology groups could be identified that correspond to three distinct strains: *Streptococcus constellatus, Streptococcus intermedius and Streptococcus anginosus* [4]. Phenotypically, the members of this group are characterized by their microaerophilic or anaerobic growth requirement, the formation of minute colonies and the frequent presence of characteristic caramel-like smell when cultured in agar plate.

The "*Streptococcus milleri*" group (SMG) are commensal organisms commonly isolated from the mouth, oropharynx, gastrointestinal tract and vagina, but they can cause a variety of human and animal infections [5]. Unlike other viridans streptococci, SMG

species are often associated with bacteremia and abscess formation. However, the pathogenic mechanisms of SMG are not yet completely understood. The frequent presence of polysaccharide capsule may help these pathogens to escape from being phagocytosed before adhering to the site of tissue damage. The production of extracellular enzymes including hyaluronidase, deoxyribonuclease, ribonuclease, gelatinase and collagenase by these organisms may contribute to its pathogenicity by degradation of connective tissues. SMG have also been observed to release extracellular products with immunosuppressive effects which may allow the organisms to survive within an abscess [6].

After "*Streptococcus milleri*" was first isolated from oral infections, subsequent studies confirmed its role in the pathogenesis of oral and teeth infection as well as its cariogenic potential [7]. In a former study investigating the bacteriology of dental abscesses, out of the ten patients, "*Streptococcus milleri*" was grown from the dental aspirate in 2 patients. One had nearly pure growth while the other one had predominant growth mixed with other oral anaerobes [8]. In a more recent study on head and neck infection by "*Streptococcus milleri*" in the paediatric patients, up to 30% of the cases have dental origin and 60% of these dental infections have local extension requiring surgical intervention [9]. Although dental infection or abscess caused by SMG are less commonly encountered nowadays probably related to better dental care, odontogenic infection should still be considered in patient with SMG bacteremia of unknown source, especially in patients with poor dental hygiene.

There is a propensity for SMG to form abscess and cause invasive pyogenic infection, including head and neck infection, brain abscesses, intra-thoracic and intra-abdominal infections. Predisposing or underlying conditions noted in most of reported cases of *S. milleri* infection include previous surgery, trauma, diabetes, immunodeficiency, malignancy, and prosthetic device. Different species of SMG, however, have different tendency to abscess formation. In order to avoid the difficulty in the charateristization of the 3 species by phenotypic tests, a study was designed to investigate the clinical importance of the three SMG species by using 16S rRNA sequence [10]. In this study, *S. constellatus* and *S. anginosus* are isolated from clinical specimens with approximately the same frequency, which is 4 times more often than *S. intermedius*. Majority (48%) of the isolates was recovered from exudates, aspirate or fluid samples, and 27% were recovered from blood samples. *S. intermedius and S. constellatus* were found to be more likely to cause abscess (86% and 73% respectively) than was *S. anginosus* (19%). Abscesses caused by *S. intermedius* tended to be associated with haemotogenous spread or were deep-seated, whereas those due to *S. constellatus* were more often superficial but seemed to cause a broader range of infection, including odontogenic and intra-abdominal disease. Moreover, while abscesses caused by *S. constellatus or S. anginosus* were more likely to be polymicrobial, *S. intermedius* was found as a solitary isolate causing abcesses in most patients. The author suggested that although *S. intermedius* was least commonly isolated species in the study, it is the most pathogenic species within the SMG.

Apart from pyogenic infection, infective endocarditis has been reported in a substantial proportion of patients with SMG bacteremia in older studies [11]. However, endocarditis becomes less commonly identified in recent series [12,13]. In a study of endocarditis caused by beta-haemolytic streptococci and *Streptococci milleri* [14], 71% of the twenty-nine patients with SMG endocarditis had underlying heart diseases. Congestive heart failure and extracardiac complications including arterial emboli and mycotic aneurysm were noted in 43% and 33% respectively. Mitral valve was found to be the most commonly involved (59%)

based on echocardiogram assessment, followed by aortic valve (45%) and tricuspid valve (7%). Intracardiac abscess formation was detected in 10% of cases. 62% of patient required cardiac surgery. The overall mortality of patient with SMG endocarditis in the study was 14% and the mortality increased to 33% in patients with prosthesis and 25% in elderly patients older than 65 years old. Among the members of SMG, it seems that *S. anginosus* is more likely to cause endocarditis when compared to the other two species. In a local study of "*Streptococcus milleri*" endocarditis, 6 cases of "*S. milleri*" endocarditis were encountered out of 377 cases of infective endocarditis diagnosed by Duke's criteria over the 5 year study period. All these "*S. milleri*" isolates were identified as *S. anginosus* by 16S ribosomal RNA sequencing [15]. The propensity of *S. anginosus* to cause infective endocarditis is supported by a mouse model study which showed, among 3 species of SMG, *S. anginosus* had a higher chance to cause endocarditis and the bacterial density was also found to be higher in vegetation caused by *S. anginosus* [16].

In conclusion, SMG has a tendency to cause abscess formation but different species have different propensity of pyogenic infection. As the three species of SMG are also associated with different clinical syndromes, identification to species level can help us to decide on the further investigation and possible source of infection.

References:

1. Guthof O: Uber pathogene "vergrunends Streptokokken". Streptokokken-Befunde bei dentogenen Abszessen und infiltraten im bereich der Mundhohle. Zentralblatt fur Bakteriologie, Parasitenkunde, Infektionskrankheiten, Hygiene 1956; 166: 553-564.
2. Colman G, Williams REO. Taxonomy of some human viridans streptococci. In: Wannamaker LW, Matsen JM (ed): Streptococci and streptococcal diseases: recognition, understanding, and management. Academic Press, New York, 1972, P. 281-299.
3. Coykendall AL, Wesbecher PM, Gustafson KB. *Streptococcus milleri, Streptococcus constellatus*, and *Streptococcus intermedius* are later synonyms of *Streptococcus anginosus*. International Journal of Systematic Bacteriology 1987; 37: 222-228.
4. Whiley RA, Hardie JM. DNA-DNA hybridization studies and phenotypic characteristics of strains within the *Streptococcus milleri* group. Journal of General Microbiology 1989; 135: 2623-2633.
5. Ruoff KL. *Streptocouus anginosus* ("*Streptococcus milleri*"): The unrecognized pathogen. Clin. Microbiol. Rev. 1988; 1: 102-108.
6. Arala-Chaves MP, Porto MT, Arnaud P, et al. Fractionation and characterization of the immunosuppressive substance in crude extracellular products released by *Streptococcus intermedius*. J Clin Invest. 1981 Jul; 68(1): 294–302.
7. Drucker, D. B., and R. M. Green. The relative cariogenicities of *Streptococcus milleri* and other viridans group streptococci in gnotobiotic hooded rats. Arch. Oral Biol. 1978; 23: 183-187.
8. Williams BL., McCann GF., Schoenknecht FD. Bacteriology of dental abscesses of endodontic origin. Journal of Clinical Microbiology. 1983; 18(4): 770-774.
9. Han JK, Kerschner JE. *Streptococcus milleri*: an organism for head and neck infections and abscess. Archives of Otolaryngology -- Head & Neck Surgery. 2001; 127(6): 650-654.
10. Clarridge JE III, Attori S, Musher DM, et al. *Streptococcus intermedius*, *Streptococcus constellatus*, and *Streptococcus anginosus* (the *Streptococcus milleri* group) are of different

clinical importance and are not equally associated with abscess. Clin Infect Dis. 2001; 32: 1511-1515.

11. Gossling J. Occurrence and pathogenicity of the *Streptococcus milleri* group. Rev Infect Dis. 1988; 10: 257-285.
12. Bert F, Bariou-Lancelin M, Lambert-Zechovsky N. Clinical significance of bacteremia involving the "*Streptococcus milleri*" group: 51 cases and review. Clin Infect Dis. 1998; 27: 385-387.
13. Nigel C. Weightman, Michael R.D. Barnham, Marion Dove. *Streptococcus milleri* group bacteraemia in North Yorkshire, England (1989-2000). Indian J Med Res. 2004; 119(Suppl): 164-167.
14. Lefort A., Lortholary O., Casassus P., et al. beta-Hemolytic Streptococci Infective Endocarditis Study Group. Comparison between adult endocarditis due to beta-hemolytic streptococci (serogroups A, B, C, and G) and Streptococcus milleri: a multicenter study in France. Archives of Internal Medicine. 2002; 162(21): 2450-6.
15. Woo PCY, Tse H, Chan KM, et al. "*Streptococcus milleri*" endocarditis caused by Streptococcus anginosus, Diagnostic Microbiology and Infectious Disease. 2004: 48(2): 81-8.
16. Kitada, K., Inoue, M., and Kitano, M. Experimental endocarditis induction and platelet aggregation by *Streptococcus anginosus, Streptococcus constellatus*, and *Streptococcus intermedius*. FEMS immunology and Medical Microbiology. 1997, 19: 25-32.

Animal bites and infections

K. Y. Tsang, K. H. Szeto , T.Y. Wong, Infection Control Branch, Centre of Health Protection I

Animal bites are commonly encountered in emergency settings. The vast majority of animal bites are caused by dogs and cats. The most common complication of an animal bite is infection. The associated pathogens can be either from animal mouth flora (aerobic and anaerobic pathogens), environmental source (*Clostridium tetani*) or victims' skin flora. Some bite wounds can be serious, causing injury and permanent disability. Bite wounds to upper limbs and hand & neck region might result in serious complications because of the close proximity to the underlying vital organs.

The acronym HELICOPTER (History, Examination, Liberal cleansing, Irrigation, Culture consideration, Operative cleansing and closure, Prophylactic antibiotics, Tetanus immunization, Elevation, Rabies risk assessment) is helpful for recalling the principles of bite wound management.[2]

1. Detailed history should include the circumstances in which the bite was sustained; the size, species, and current location of the biting animal; whether the bite was provoked or apparently unprovoked; the time elapsed in hours since the wound was inflicted; and the immune status and current medications of the victims sustaining the bite.

2. Physical examination should focus on any neurovascular deficit and signs of infection. Radiographs should be performed if suspecting fracture, foreign body, or penetration of a bone or joint.

3. All wounds should be cleansed liberally with copious irrigation. Remove foreign bodies and necrotic tissue if present.

4. Delayed suturing in the operating room under general anaesthesia is advised for contaminated, large or deep wounds and hand wounds.

5. Taking wound culture is indicated if there is evidence of infection.

6. Prophylactic use of antibiotics for

apparently uninfected wounds is another controversial issue unsettled. One meta-analysis of eight randomized trials found an absolute benefit of one infection prevented for every 14 patients treated. [6] Antibiotic prophylaxis should generally be administered in the following high risk situations: facial, hand, genital bite wounds, deep puncture wounds, wounds requiring surgical debridement, older patients, immunocompromised patients, a bite wound near or in a prosthetic joint, bite wound in an extremity with underlying venous and/or lymphatic compromise (eg, following mastectomy). [6,7]. Amoxicillin-clavulanate (IV 1..2g Q8H; PO. 1g BD) is the treatment of choice [8]. Among patients with a history of immediate-type hypersensitivity to penicillins or other beta-lactam antibiotics or delayed reactions (rash) to penicillins, alternative options include clindamycin (600 mg IV, then 300 mg PO TDS) plus an oral fluoroquinolone (ciprofloxacin 500 mg BD or levofloxacin 750 mg daily or moxifloxacin 400 mg daily) in adults or clindamycin plus trimethoprim-sulfamethoxazole in children. [9]

7. Tetanus-prone wounds include those wounds complicated by delay in treatment for over 6 hours, deeply punctured and heavily contaminated. [1] A review of the tetanus status of the victim should be performed. Active tetanus immunization with tetanus toxoid (Td) should be given to those who have completed the primary immunization but have not received a booster immunization in the past five years. For bite victims who have received less than three doses of tetanus toxoid or whose immunization status is uncertain, a dose of tetanus toxoid (0.5 mL intramuscularly) and tetanus immune globulin (250 U intramuscularly) should be considered in patient with tetanus-prone wounds.[9]

8. Wound elevation is particularly important for hand and leg injuries for minimizing edema formation.

9. Rabies risk should be assessed. A healthy dog and cat should be observed for ten days; if the animal is ill at the time of the attack or develops illness during the period of observation, then prompt evaluation by a veterinarian should be performed to determine the need for animal sacrifice to evaluate for rabies. Rabies prophylaxis is administered if the dog or cat is rabid or suspected rabid. 5 doses of full course active immunization of human diploid cell vaccine (1 mL intramuscularly on days 0, 3, 7, 14, and 28) is recommended. Besides, one dose of human rabies immune globulin (HRIG) should be given intramuscularly (20 IU/kg) on the first day if the animal is highly suspicious of being rabid. If anatomically feasible, up to one-half of the dose should be infiltrated around the wounds. If the animal remains healthy during the ten-day observation, then individuals bitten need not be vaccinated. Full course of active immunization should be offered if the animals could not be captured for observation. [1]

10. An estimated 15% to 20% of dog bites and 50% of cat bites, for which medical intervention is sought, become infected. Practically, a handful of microbial species need to be considered as potential pathogens when one is considering empirical treatment. [2]

Specific pathogens associated with animal bites

Dog and Cat bites

Alpha haemolytic Streptococci are the pathogens most commonly isolated from infected dog bites, but *Staphylococcus aureus* (20-30%) can also be isolated from 20% to 30% of these wounds. *Staphylococcus intermedius* is a newly

recognized pathogen as well. It may be coagulase positive, requires differentiation from *S. aureus*, and is penicillin-susceptible. *Eikenella corrodens*, anaerobic Gram-negative bacillus, is also a quite common isolate from dog bite wound It has been implicated in a number of sinopulmonary infectious processes.

Pasteurella species were the most common bacteria from dog and cat bites (50 and 75 percent respectively); *P. canis* was most common from dog bites, whereas *P. multocida* and *P. septica* were most frequent from cat bites. Infection caused by *P. multocida* is characterized by a rapid onset of intense cellulitis, often within hours or a day after the wound, and with serosanguineous or seropurulent discharge. [9,10]

Capnocytophaga canimorsus, part of normal flora of dogs and cats, is a Gram-negative bacillus that can result in fatal infection, characterized by septic shock and disseminated intravascular coagulopathy in individuals with functional or anatomic asplenia, hepatic dysfunction, or immunosuppression. In a case series of endocarditis caused by *Capnocytophaga*, one-third of the patients had a history of known antecedent dog bite; but no patient had splenectomy. The clinical presentations were mostly subacute; and the mortality with this condition was reported to be to 25% [11]. Another report described symmetrical peripheral gangrene in patients with *C. canimorsus* septicaemia and prompted early diagnosis in patients who became severely ill after a dog bite. Other complications include renal failure, pulmonary infiltrates, arthritis, and meningitis. In around 40% of the cases reviewed however, no risk factors nor history of animal bites could be identified. [12]

Bartonella henselae, a curved Gram-negative rod, is the aetiologic agent of cat scratch disease (CSD) characterized by regional lymphadenopathy and disseminated infections (visceral organ, neurologic, and ocular involvement). Patients with localized disease generally have a self-limited illness, whereas those with disseminated disease can have life-threatening complications. It can cause disease in both normal and immunocompromised hosts. 80 percent of cases occur in children. *B. henselae* infection should be considered in the children with prolonged fever. Antibiotic should only be indicated for immunocompromised host or patients with severe and disseminated infections. Infections caused by Bartonella henselae are generally resistant to penicillin and the recommended choice of antibiotics should include septrin, fluoroquinolone, rifampicin and gentamicin. [5]

Rodent / Rat bites

Rodents transmit a variety of infections, including leptospirosis, hantavirus, and plague, but disease in humans usually arises from contact with animal excreta, such as urine, or indirectly through an insect vector such as the flea, rather than from a bite.

Four to 10 percent of patients bitten by rats acquire rat bite fever (RBF). *Streptobacillus moniliformis* (North USA) and *Spirillum minus* (Asia) are the recognized pathogens of causing the illness. The symptoms of RBF caused by *S. moniliformis* start abruptly 2-10 days following exposure with fever, myalgias, arthralgias, vomiting, and headache. The fever is often irregularly relapsing. The initial symptoms are followed by a maculopapular rash on the extensor surface of the extremities and may involve the palms and soles. Subsequently, up to 50% of patients may experience polyarthritis [13,14]. In contrast, RBF caused by S. minus, has a longer incubation period (1-3 weeks), and the initial wound may reappear at the onset of the systemic illness or persist with aedema and ulceration. Arthritis is not a common clinical finding. [15]

Antimicrobial regimens for established bite wound infection [2,3,4,9,10,16]

Antibiotic therapy should be directed against

the polymicrobial infection (including aerobes and anaerobes species) that frequently occurs following animal bite wounds. The combination of beta-lactam and beta-lactamase inhibitor is widely recommended for patients with no history of penicillin allergy. Amoxicillin-clavulanate (1.2 g IV Q8H) is an appropriate regimen, while Amoxicillin-clavulanate (875/125 mg PO BD) should be effective in patients who can be switched to oral medications to finish therapy. It can also be given as initial therapy if parenteral antibiotics are not needed.

For patients with history of allergic reaction towards beta-lactam antibiotics, combinations of clindamycin (600 mg IV Q8H/ 300 mg PO TDS) with either doxycycline(100 mg PO BD for patients >12 y.o) or fluoroquinolone (ciprofloxacin 400 mg IV Q12H /500 mg PO BD or levofloxacin 750 mg PO daily or moxifloxacin 400 mg PO daily), or trimethoprim-sulfamethoxazole are recommended. Treatment is usually administered for at least 10-14 days, and extended if there are complications such as septic arthritis or osteomyelitis.

Table 1. An acronym for management of animal bite wounds

H	History
E	Examination
L	Liberal Cleansing
I	Irrigation
C	Closure and culture consideration
O	Operative cleansing and closure
P	Prophylactic or therapeutic antimicrobial agent use
T	Tetanus immunization status
E	Elevation
R	Rabies risk assessment

Table 2. Some of the pathogens associated with animals bites

Animal	**Pathogens**
Mammal	Rabies
Dog	*α-haemolytic Streptococci* *Staphylococci aureus and intermedius* *Eikenella corrodens* *Capnocytophaga canimorsus*
Cat	*Bartonella henselae* *Pasteurella multocida* *Francisella tularensis*
Macaque	*Herpesvirus simiae (B virus)*
Rat	*Streptobacilus moniliformis* *Spirillum minus*
Fresh-water species	*Aeromonas hydrophila* *Mycobacterium marinum*
Salt-water species	*Vibrio vulnificus* *Mycobacterium marinum*

Table 3. Polymicrobial organisms isolated from dog/ cat bite wounds

Aerobes	**Anaerobes**
α-haemolytic *Streptococcus*	Actinomyces
Staphylococcus aureus and *intermedius*	*Bacteroides*
Pasteurella multocida	Fusobacterium
Moraxella species	*Peptostreptococcus*
Corynebacterium species	*Prevotella*
Neisseria species	*Capnocytophaga species*
	Eikenella corrodens

References:

1. A&E clinical guidelines on management of rabies and tetanus infection from HAHO website. Accessed at http://aeis.home/AEMedGuide/aeguide.html on 8 Aug, 2008.
2. Morven S. Edwards. Animal Bites: A Practical Approach to Management. Clinical Updates in Paediatric Infectious Disease Volume II, Issue 5 - March 1998
3. Companion animals and human health risk: Animal bites and rabies. Accessed at http://www.medscape.com on 26 March 2008
4. Animal bites from Merck Manual professional. Access on http://www.merck.com/mmpe/index.html.
5. Bartlett JG. Bartonella species. Accessed at http://www.hopkins-abxguide.org on 26 March, 2008
6. Cummings P . Antibiotics to prevent infection in patients with dog bite wounds: a meta-analysis of randomized trials. Ann Emerg Med 1994 Mar;23(3): 535-40.
7. Fleisher GR. The management of bite wounds. N Engl J Med 1999; 340:138
8. Goldstein EJ. Bite wounds and infection. Clin Infect Dis 1992 Mar;14(3): 633-8.
9. Baddour LM. Soft tissue infections due to dog and cat bites in adults. Accessed at http:// www. UpToDate.com on 26 March, 2008
10. Endom EE. Animal and human bites in children Accessed at http:// www. UpToDate.com on 26 March, 2008
11. Sandoe JA. Capnocytophaga canimorsus endocarditis. J Med Microbiol 2004; 53; 245-8
12. Kullberg BJ, Westendorp RG, van 't Wout JW, et al. Purpura fulminans and symmetrical peripheral gangrene caused by Capnocytophaga canimorsus (formerly DF-2) septicemia--a complication of dog bite. Medicine (Baltimore) 1991; Vol. 70; pp. 287-92
13. Stehle P, Dubuis O, So A, Dudler J. Rat bite fever without fever. Ann Rheum Dis 2003 Sep;62(9):894-6.
14. Tandon R, Lee M, Curran Ex et al.A 26-year-old woman with a rash on her extremities. Clin Infect Dis. 2006 Dec 15;43(12):1585-6, 1616-7.
15. Trucksis M. Rat bite fever. Accessed at http:// www. UpToDate.com on 26 March, 2008
16. Bartlett JG. Bite Wounds. Accessed at http://www.hopkins-abxguide.org on 26 March, 2008

An unusual presentation of *Clostridium difficile* colitis

Kitty Fung, Department of Pathology, United Christian Hospital

Case presentation

The patient was a 57 years old man with history of post-traumatic spinal surgery (L2/3) in 1994, resulted in cauda equina syndrome, neurogenic bladder, laxative dependent bowel opening and bilateral lower limb numbness. He was hospitalized in November 2007 because of a difficult-to-heal right calf wound which began after a minor scratch. Several wound specimens grew *Pseudomonas aeruginosa.* He was put on multiple antibiotics including piperacillin, gentamicin and ciprofloxacin at various stages during his course of illness. Skin grafting failed initially despite repeated wound dressings and surgical debridement. However, the wound slowly improved and a second skin grafting was planned. Another course of intravenous piperacillin was started when the Gram's smear revealed

heavy amount of pus cells and culture yielded heavy growth of *Pseudomonas aeruginosa.* Wound debridement and skin graft were performed 1 week later. On day 2 after the operation, the patient developed fever which persisted at around 38.5 to 39.5°C. The skin graft was well taken. He was not septic looking and had no other physical complaint.

Examination of his cardiovascular, respiratory and abdominal systems was unremarkable. Initial investigations including a chest radiograph and ultrasound scan of the abdomen were unremarkable. There was marked peripheral leukocytosis (up to 38.5 x 10^9 /L, 94% neutrophils). C-reactive protein (CRP) was also raised (up to 378 mg/L). On day 9 after the operation, all antibiotics were stopped, and sepsis workup was repeated. Imipenem/cilastatin 500 mg every 6 hours was started the next day. An urgent computed tomography scan of the thorax down to pelvis was performed. It revealed no collection, but showed abnormal colonic wall thickenings involving the ascending colon and caecum, suggestive of colitis. With the possibility of pseudomembranous colitis, and the differential diagnosis of lymphoma. imipenem/cilastatin was stopped, and oral metronidazole 400 mg three times daily was started. Stool specimens were collected for the detection of *Clostridium difficile* toxin. Two days later, the patient started to pass yellowish loose stools, bedside faecal occult blood test was positive, and the *C. difficile* toxin assay on his fecal specimen was also positive. The patient continued to have persistent fever, passed loose stools 2-3 times a day but had no frank watery diarrhoea. Colonoscopy was performed later to look for other possible pathology. It revealed diffuse colitis with multiple small ulcers covered by fibrinous exudate and cobblestone appearance from rectum up to ascending colon. Biopsy showed that the colonic mucosa was covered by adherent "mushroom" like pseudomembrane composed of fibrin, mucus and inflammatory debris, compatible with pseudomembranous colitis. A course of oral metronidazole for a total of 14 days was completed. The fever subsided gradually, the white cell count and the CRP became normalized, and the patient was discharged.

Discussion

We reported a patient with *C. difficile* colitis presented with fever and markedly raised inflammatory markers but delayed gastrointestinal symptoms. The late onset of diarrhea in our patient might be related to his underlying conditions (laxative dependent bowel opening) or mild ileus not readily detected by ultrasound of the abdominal imaging. *C. difficile* infection (CDI) should be considered for patients with marked leukocytosis, even in the absence of diarrheal symptoms in appropriate settings [1]. Characteristic radiological and colonoscopy features may be useful for the diagnosis of CDI.

C. difficile is a Gram-positive, anaerobic spore-forming bacillus that is recognized as the principal causative agent of nosocomial diarrhea, giving a wide spectrum of disease ranging from mild diarrhea to life-threatening colitis and peritonitis. It causes approximately 15% to 20% of antibiotic-associated diarrhea and nearly all cases of pseudomembranous colitis [2]. *C. difficile* is *w*idely present in the environment. It is also present in the normal bowel flora in ~3% of healthy adults and 10% to 20% of persons aged over 65.

Disease is mediated by spores that are shed in large numbers by infected patients. Spores are resistant to common detergent and disinfectants and can survive in the environment for months. They are transmitted via faecal-oral route; e.g. via contact of contaminated hands or environment followed by ingestion. The spores germinate and the bacteria colonise the intestine, which then become overgrown when normal gut flora are suppressed by antibiotics. Clindamycin, cephalosporins and penicillins are the main inciting antibiotics, although virtually every antibiotic has been implicated. Toxinogenic strains

usually produce cellular cytotoxins, TcdA and TcdB, causing damage to colonic mucosa and formation of pseudomembrane. Human infants sometimes harbour *C difficile* in faecal flora but develop no symptom, which is generally believed to be related to immature toxin receptors. In addition to established risk factors of recent exposure to antibiotics, advanced age and prolonged stay in healthcare settings, other risk factors such as surgery to the gastrointestinal tract, gastric acid suppression, and underlying medical conditions or immunosuppression are also reported.

Recently emerged hypervirulent and highly transmissible strains with toxinotype III, REA group BI, PCR ribotype 027/NAP1 have caused geographically dispersed outbreaks in Europe and North America. The epidemic strain is characterised by better sporulation capacity [3] and resistance to fluoroquinolones. It contains the binary toxin gene and have an 18-bp deletion in the *tcdC* toxin repressor gene resulting in hyperproduction of TcdA/B [4]. This may account for the more severe disease, higher rates of complication, relapse and mortality associated with the 027 phenotype. A recent analysis of 300 established *C difficile* colitis patients revealed that approximately 69% of cases were acquired in the community or while the patient resided at a nursing home, suggestive of a shift of CDI towards the outpatient setting [5].

CDI should be considered as a cause if unexplained nosocomial diarrhea. Flexible bedside sigmoidoscopy may allow rapid diagnosis in severe cases. The optimal means of laboratory diagnosis of CDI is not yet clear. The cell cytotoxin assay is highly sensitive (94-100%) and specific (99%), and is considered as the gold standard for diagnosis. However, the test takes 48 hours to complete, is expensive and has limited availability. The enzyme immunoassays (EIA) for toxins A and/or B are faster (~4 hours) and specific, but has variable sensitivity ranging from 57% to 100%. Recent studies on new EIA kits however, revealed sensitivity and specificity of over 90% and 87% respectively when compared with cytotoxin assay [6]. Immunochromatogenic assay is a rapid test that detects glutamate dehydrogenase (GDH) and toxin A. GDH is produced in significantly higher quantities than toxin A/B. The negative predictive value of such assay could be up to 98.7% [7]. The test has significant advantages in terms of turnaround time and ease of use. However, verification is required for toxin A-negative results since both non-toxigenic and toxin B-only *C. difficile* strains may be GDH-positive. Another approach is to perform GDH screening followed by a sensitive method to confirm positives. Conventional culture of stool requires at least 72 hours. Demonstration of toxin A and/or B would be required on the cultured isolate to confirm that it is a toxigenic strain. In view of the labour intensity and its extended turnaround time, it is not routinely performed for diagnostic purposes. Polymerase chain reaction (PCR) assays have also been applied but are not ready for routine use yet. Stool assays targeting on toxin B gene has attracted great attention since finding of this gene is likely to correlate with the presence of toxin as measured by immunoassay, cytotoxicity or toxigenic culture. However any PCR on stool samples will require nucleic acid extraction, making this assay much less efficient than immunoassay.

Regarding management of CDI, for mild cases, discontinuation of the causative antibiotics alone may suffice [8]. Anti-motility agents may exacerbate colitis and should be strictly avoided. For the past 20 years, there has been little advancement in the treatment of CDI. The standard is still metronidazole (the only FDA-approved therapy for CDI), 500 mg 3 times per day, for mild to moderate diseases. Since only the 200 mg tablets metronidazole are available locally in Hong Kong, a dosage of 400 mg t.d.s. is commonly used. Oral vancomycin, 125 mg to 250 mg 4 times per day, is recommended for the initial treatment of severe, uncomplicated CDI, or when metronidazole therapy is ineffective. There

is no evidence to support combination therapy in uncomplicated CDI. High dose vancomycin, 500 mg 4 times per day, and/or intravenous metronidazole, 500 to 750 mg every 8 hours are recommended for severe and complicated CDI. Resistance to metronidazole has been rarely reported: however it is unlikely to be the case of treatment failure in most cases. Vancomycin is not absorbed in the gut and produces very high local drug concentration: therefore it is considered superior to metronidazole for severe and complicated CDI cases. It allows significant shortening of the mean duration of symptoms [9]. Delivery of vancomycin by retention enema or nasogastric tube is sometimes necessary in cases of severe ileus. Other available antimicrobial agents include teicoplanin, bacitracin, fusidic acid, rifaximin and nitazoxanide [10]. Their efficacy are, however, not entirely certain. Immunotherapies under study include use of human gamma globulin, anti–*C difficile* whe protein concentrate and a toxoid *C difficile* vaccine. For relapsing diseases, repeat oral metronidazole or vancomycin can be used. However, metronidazole should not be used beyond first recurrence or longer than 14 days. Other treatment modalities under investigation include prolonged, tapered course of vancomycin, restoration of normal intestinal flora by probiotics such as *Saccharomyces boulardii, Lactobacillus plantarum or* faecal transplant administered by faecal enema. For patients with severe CDI, timely surgical intervention and intensive care should be considered.

National guidelines consisting of a bundle of 5 main elements have been developed in the United Kingdom to reduce the risk of *C. difficile* infection/outbreaks [11]. Active surveillance of cases should be performed to identify changes in local epidemiology. Antibiotic use should be minimized to preserve the beneficial bowel flora to contain growth of *C. difficile.* Ensure vigilant hand hygiene measures, with the understanding that hand washing with soap and water is superior to alcohol-based agents that are not very effective against the spores. Contact precautions should be applied until diarrhoea has subsided for 2 days. Single room or cohort isolation for patient placement, use of dedicated equipment, gloves and gowns for patient care are required. Ward environment must be scrupulously cleansed with chlorine-releasing agent, e.g. hypochlorite (1000 ppm). Attention should be paid to most touched areas and perform terminal decontamination when the patient is discharged.

With the changes in the epidemiology of CDI and the emergence of epidemic hypertoxigenic strains, an aggressive and multi-pronged approach to early diagnosis and control is warranted.

References

1. Wanahita A, Goldsmith EA and Musher DM. Conditions associated with leukocytosis in a tertiary care hospital, with particular attention to the role of infection caused by *Clostridium difficile.* Clin Inf Dis. 2002;34:1585–1592.
2. Bartlett JG. Clinical practice. Antibiotic-associated diarrhea. N Engl J Med 2002;346:334–9.
3. Akerlund T, Persson I, Unemo M, et al. Increased sporulation rate of epidemic *Clostridium difficile* Type 027/NAP1. J Clin Microbio. 2008; 46:1530-1533.
4. MacCannell DR, Louie TJ, Gregson DB, et al. Molecular analysis of *Clostridium difficile* PCR Ribotype 027 isolates from eastern and western Canada. J Clin Microbiol. 2006; 44(6): 2147–2152.
5. Kumar V, Yoselevitz S, Dutta SK. *Clostridium difficile* colitis: A shift from a traditional hospital acquired infection to a community and nursing home-based disease. Am J Gastroenterol.

2007;102:A-266.

6. Musher DM, Manhas A, Jain P, et al. Detection of *Clostridium difficile* toxin: comparison of enzyme immunoassay results with results obtained by cytotoxicity assay. J. Clin. Microbiol.*2007;* 45: 2737-2739.

7. Turgeon DK, Novicki TJ, Quick J, et al., Six rapid tests for direct detection of *Clostridium difficile* and its toxins in fecal samples compared with the fibroblast cytotoxicity assay, J Clin Microbiol, 2003;41:667–70.

8. Teasley DG, Gerding DN, Olson MM, et al. Prospective randomised trial of metronidazole versus vancomycin for *Clostridium difficile*-associated diarrhoea and colitis. Lancet 1983;2:1043–6.

9. Wilcox MH, Howe R. Diarrhoea caused by *Clostridium difficile*: response time for treatment with metronidazole and vancomycin. J Antimicrob Chemother 1995;36:673–9.

10. Bricker E, Garg R, Nelson R, et al. Antibiotic treatment for *Clostridium difficile*-associated diarrhea in adults. Cochrane Database Syst Rev 2005(1):CD004610.

11. Health Protection Agency, United Kingdom. A good practice guide to control *Clostridium difficile*. Jan 2007. http://www.hpa.org.uk/web/HPAwebFile/HPAweb_C/1194947384014 (accessed on 21 May 2008)

An HIV-infected patient with Gram-negative bacilli bacteraemia

W. M. Hui, K. W. Choi, Department of Medicine, Alice Ho Miu Ling Nethersole Hospital

Case presentation

We present a case of gram-negative bacteraemia involving an HIV-infected patient. This was a 48 year-old gentleman, a Hong Kong resident, who resided in Guangzhou. One year prior to his presentation, he started to lose weight. Eight months later, he began to produce intermittent, blood-stained sputum. His past health had been good all along, except for an episode of "infectious mononucleosis-like" illness in 2003, which was reported to consisted of fever, sore throat and a generalized skin rash.

On presentation, he had a body temperature of 38.7^{o}C and a blood pressure of 90/50mmHg. Peripheral oxygen saturation measured by pulse oximetry was 97% in room air. Physical examinations of the respiratory, abdominal, cardiovascular and neurological systems were unremarkable. There was no skin lesion, genital ulcer or palpable lymph node. Complete blood count revealed leucopenia (3.2x10^{9}/L), with a decreased lymphocyte count of 0.5x10^{9}/L. There was also normocytic normochromic anaemia with a haemoglobin of 8.0g/dL. The platelet count was normal. INR was 1.4. Serum creatinine and liver function tests were within normal limits. Chest radiograph showed a right apical cavitatory lesion.

Empirical intravenous amoxicillin-clavulanate was started to cover for possible community-acquired pneumonia. Sputum was collected for bacterial culture, acid-fast bacilli (AFB) smear, mycobacterial culture, and cytological examination. However, the patient subsequently developed septic shock and was admitted to the Intensive Care Unit. The antibiotic regime was changed to ceftriaxone, and the patient's haemodynamic status was gradually stabilized together with supportive therapies.

Oral candidiasis was noticed. On further questioning, the patient disclosed that he had been tested positive for HIV in mainland China. HIV serology was repeated in our unit and was found to be positive by both ELISA and immunoblot methods. Bacterial culture and repeated AFB smears of sputum were negative. However, his blood culture subsequently grew a gram-negative bacilli which was susceptible to ciprofloxacin and cefotaxime, but was resistant to ampicillin, chloramphenicol,
trimethoprim-sulfamethoxazole and nalidixic acid. Intravenous ceftriaxone was continued for a total of two weeks. The patient remained afebrile and the right apical cavitatory lesion was also slowly resolving. The blood culture isolate was identified to be a Group C *Salmonella*. Other than the right apical pneumonia, there was no evidence of bone and joint involvement or endocarditis. A contrast CT scan was performed, which showed no mycotic aneurysm. His CD4 T-lymphocyte count was 24 cells/μL, and oral trimethoprim-sulfamethoxazole was started for primary prophylaxis against *Pneumocystis jiroveci*. The patient was then evaluated for initiation of antiretroviral therapy.

Discussion

Salmonella species are gram-negative, enterobacteriaceae. Non-typhoidal salmonella (NTS) infections is a common cause of self-limiting gastroenteritis. However, life-threatening diseases such as bacteraemia and extra-intestinal complications may also occur, particularly in immunocompromised patients.

NTS are common commensals organisms in a wide range of domestic pets and wild animals, including reptiles, birds, dogs, cats and rodents. Human infections can be transmitted through ingestion of contaminated food or water. Foodborne outbreaks are not uncommonly reported, with contaminated eggs, dairy products, poultry and meat being implicated as the sources of infection. *S. typhimurium* and *S.enteritidis* are the two most frequentlyly isolated serotypesn associated with clinical infections [1].

Regarding its pathogenesis, the bacteria enter the intestinal epithelial cell through endocytosis. Thereafter, a cell-mediated immune response involving interleukin (IL)-12 .and IL-23 sets in. These cytokines stimulate the production of interferon (IFN)-γ, tumor necrosis factor (TNF)-α and granulocyte-macrophage colony-stimulating factor (GM-CSF) by T-helper cells and NK cells, which, in turn, activate the macrophages for bacterial killing [2]. Patients with T-cell dysfunction have impaired clearance of the bacteria and are prone to severe/disseminated *Salmonella* infections.

Risk factors for developing severe or disseminated NTS infections include corticosteroid use, chemotherapy, lymphoma, chronic lymphocytic leukaemia, organ transplantation, and HIV/AIDS [3]. Besides bacteremia, other extra-intestinal infections may include endocarditis, arteritis, osteomyelitis, septic arthritis, urinary tract infection and soft tissue infection.[4] Recurrent *Salmonella* bacteraemia is required as one of the AIDS-defining illnesses. Morbidity and mortality remains high even appropriate antibiotics are given.

Diagnosis of NTS infections relies on a positive bacterial culture, usually from stool, urine and blood. Further specimen collection depends on the clnical evaluation/suspicion of a focal infection focus. Repeated blood culture should be taken: the risk of extra-intestinal infections is substantially higher in patient with high grade/persistent bacteraemia.

Antibiotic treatment is generally not indicated to treat uncomplicated salmonella gastroenteritis in immunocompetent patients, since it is usually self-limiting. However, in patients who are immunocompromised, antibiotic therapy is recommended because of the relatively high risk of bacteraemia and extra-intestinal infections. Fluoroquinolone,

e.g. ciprofloxacin, given for 7 to 14 days for those with mild gastroenteritis without bacteraemia and 4 to 6 weeks for those with bacteraemia and low CD4 T-lymphocyte count <200/mm^3 is recommended.[5] However, drug resistant NTS is emerging. Strains of *S. typhimurium* resistant to chloramphenicol, ampicillin, sulphonamides, streptomycin and tetracycline have been documented in the USA with increasing prevalence.[6] In Taiwan, it was shown in 2003 that 44% of NTS isolates (serotypes were not specified) were resistant to ampicillin, 49% resistant to chloramphenicol, and 31% resistant to trimethoprim-sulfamethoxazole.[7] In Hong Kong, 2.5% of salmonella isolated (including serotypes *S. enteritidis*, *S. typhimurium*, *S. derby*, *S. muenster*, *S. typhi*, etc.) at Public Health Laboratory Centre between 2000 and 2005 showed intermediate susceptibility or resistance to ciprofloxacin.[8]

Current NCCLS MIC breakpoints of ciprofloxacin are ≥4μg/ml (resistant) and ≤1μg/ml (susceptible),[9] but treatment failure has been observed in NTS infection caused by strains with raised MICs of ciprofloxacin which was still within the 'susceptible' range. Compared with nalidixic acid-susceptible NTS, nalidixic acid-resistant NTS has been shown to have higher MICs of ciprofloxacin within the upper part of susceptible range 0.12-0.5μg/ml and associated with higher rates of clinical treatment failure.[9] Therefore, patients who are infected by NTS with raised MICs of ciprofloxacin should be monitored closely for treatment failure if they are put on ciprofloxacin therapy. Alternative antibiotics such as extended spectrum cephalosporin (e.g. ceftriaxone or cefotaxime) according to susceptibility should be considered.[5] Prevalence of ceftriaxone or cefotaxime-resistant NTS is still generally low at 0-5%. For instance, it was 1.5% in Taiwan according to data in 2003.[7] Trimethoprim-sulfamethoxazole is also recommended in the latest guideline of Infectious Disease Society of America as an alternative to fluoroquinolone. However, in areas where the prevalence of trimethoprim-sulfamethoxazole resistant NTS is high [7], macrolides like azithromycin may be a more reasonable alternative. The prevalence of azithromycin-resistant NTS currently remains low at 0-5% in Thailand and Vietnam.[10] However, a word of caution is that in one report, there was no significant difference in the eradication of NTS carriage between azithromycin and placebo.[11] Furthermore, there is no controlled trial for azithromycin treatment against active NTS infection.

Treatment duration for focal infections depends on the infection site involved and the clinical response. Surgical interventions may be necessary for endovascular, bone and joint infections. Long term maintenance therapy is necessary for HIV-infected patients with recurrent NTS bacteraemia. Choice of antibiotics for secondary prophylaxis depends on the antibiotic susceptibility. If susceptible, a fluoroquinolone like ciprofloxacin is recommended.[12] Zidovudine may have a bactericidal effect on *Salmonella* organisms. A lower rate of recurrence of NTS has been shown in patients who were given zidovudine with or without combination of ciprofloxacin or trimethoprim-sulfamethoxazole, independent of CD4 T-lymphocyte count.[13] However, evidence is not sufficient to support the standard use of zidovudine in HIV-infected patients with salmonellosis. Apart from prophylactic chemotherapy, patients should be advised to avoid intake of raw or undercooked food, also should wash their hands after handling of pets, and avoid contact with reptiles or animals with diarrhea to prevent bacterial enteric infections.[12]

References:

1. Olsen SJ, Bishop R, Brenner FW, et al. The changing epidemiology of Salmonella: trends in serotypes isolated from humans in the United States, 1987–1997. J Infect Dis. 2001 March;183(5):753-61.
2. MacLennan C, Fieschi C, Lammas DA, et al.

Interleukin(IL)–12 and IL-23 are key cytokines for immunity against Salmonella in humans. J Infect Dis. 2004 Nov;190(10):1755-7.

3. Shimoni Z, Pitlik S, Leibovici L, et al. Salmonella bacteraemia: age-related differences in clinical presentation, bacteriology, and outcome. Clin Infect Dis. 1999 Apr;28(4):822-7.
4. Fisker N, Vinding K, Mølbak K, et al. Clinical review of non-typhoid Salmonella infections from 1991 to 1999 in a Danish country. Clin Infect Dis 2003 Aug;37(4):e47-52
5. US CDC. Treating Opportunistic Infections among HIV-infected adults and adolescents. MMWR. 2004;53(RR-15)
6. Glynn MK, Bopp C, Dewitt W, et al. Emergence of multidrug-resistant *Salmonella enterica* serotype Typhimurium DT104 Infections in the United States. N Engl J Med. 1998 May;338(19):1333-8
7. Su LH, Chiu CH, Chu C, et al. Antimicrobial resistance in nontyphoid *Salmonella* serotypes: a global challenge. Clin Infect Dis. 2004 Aug;39(4):546-51
8. Chu YW et al. Quinolone resistance determinant qnrA3 in clinical isolates of *Salmonella* in 2000–2005 in Hong Kong (Letter). J Antimicrob Chemother 2006 Oct;58(4):904-5
9. Crump JA, Barrett TJ, Nelson JT, et al. Reevaluating fluoroquinolone breakpoints for *Salmonella enterica* serotype typhi and for non-typhi Salmonellae. Clin Infect Dis. 2003 July;37(1):75-81
10. Isenbarger DW, Hoge CW, Srijan A, et al. Comparative antibiotic resistance of diarrhoeal pathogens from Vietnam and Thailand, 1996-1999. Emerg Infect Dis. 2002 Feb;8(2):175-80.
11. Sirinavin S, Thavornnunth J, Sakchainanont B, et al. Norfloxacin and azithromycin for treatment of nontyphoidal salmonella carriers. Clin Infect Dis. 2003 Sep;37(5):685-91
12. US CDC. Guidelines for preventing opportunistic infections among HIV-infected persons. MMWR. 2002;51(RR-8)
13. Casado JL, Valdezate S, Calderon C, et al. Zidovudine Therapy Protects against *Salmonella* bacteraemia recurrence in Human Immunodeficiency Virus–Infected patients. J Infect Dis. 1999 Jun;179(6):1553–6