CANADIAN BACTERIAL SURVEILLANCE NETWORK

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Methicillin-resistant *Staphylococcus aureus* (MRSA) As A Cause Of Infections In Domestic Animals: Evidence For A New Humanotic Disease?

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is an increasingly common cause of disease. It was initially described as a human nosocomial isolate, and this remains its most common environmental niche. More recently, it has been reported in community populations, especially affecting people exposed to hospitals and similar institutions. The total incidence of reported human MRSA cases in Ontario has risen 14-fold in five years, from 586 in 1994 to 8222 in 1999.

Despite its predominance in humans, MRSA has infrequently been identified as a pathogen in other species. It has been reported in several domestic animals including canine, bovine and equine populations; however, these reports usually described cases arising from nosocomial settings. Some of these studies have suggested that MRSA may be spread from human caregivers to animals. The purpose of this study was to describe cases of MRSA infection in animals possibly derived from community sources, and to identify potential risk factors for its transmission between humans and animals.

Isolates were submitted by community veterinarians to our laboratories from July to December 2000 for speciation and antibiotic sensitivity testing. All *S. aureus* isolates were initially tested on Microscan for antibiotic sensitivities. Isolates flagged as oxacillin resistant were then confirmed by the Kirby-Bauer method. The human families of animals with confirmed MRSA colonization or infection were sent questionnaires detailing risk factors and medical histories for all animals and humans in the household. Genomic DNA from the MRSA isolates was digested by SmaI and analysed by pulsed-field gel electrophoresis (PFGE).

MRSA Case 1: A 47 year old male human underwent numerous hospitalizations from August to December 1999 for evaluation and surgical treatment of testicular cancer. The patient recovered uneventfully. His 9 year old Bishon Frise female dog had a left eye cyst removed as an outpatient procedure in January 2000. Despite the use of entamicin and cephalexin post-operatively, the surgical site became infected two days later. It initially responded to treatment with amoxicillin-clavulanate and tobramycin, but a repeat episode occurred in June 2000. MRSA grew from an eyelid swab from the dog.

Flouroquinolone resistance in *Streptococcus pneumoniae*

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The emergence of *Streptococcus pneumoniae* resistant to penicillin and the macrolide antibiotics has raised concerns regarding the use of these antimicrobials as empiric agents for the treatment of lower respiratory tract infections (1). Fluoroquinolones (FQs) with increased activity against *S. pneumoniae*, such as levofloxacin, moxifloxacin, and gatifloxacin (respiratory FQs), are now available in many countries where they are used for the treatment of patients with pneumonia who are at risk for infection due to multidrug-resistant strains (1).

The FQs are potent antibacterial agents that have DNA gyrase (GyrA) and DNA topoisomerase IV (ParC) as their intracellular targets. In *S. pneumoniae,* different FQs have been shown to have different preferential target binding sites. For levofloxacin and ciprofloxacin, the preferential target is ParC whereas for gatifloxacin and moxifloxacin it is GyrA (2). A mutation in the preferential target (first-step mutation) decreases its binding affinity and typically results in a decrease in the susceptibility of the organism to the fluoroquinolones. Decreased affinity to the preferred target results in increased binding of the FQ to the second target (3). A second-step mutation in this alternative target results in a further reduction of susceptibility of the organism (4).

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MRSA Case 2: A 7 year old female Boxer dog developed an ear infection in July 2000, and subsequently had orthopaedic knee surgery later that same month. It is uncertain whether the animal had been hospitalized for any pre-operative evaluations prior to developing the infection. Initial ear swabs were positive for MRSA. Despite courses of antibiotic treatment, the dog continued to have chronic outer ear infections for several months. None of the humans in the household reported any major medical illnesses or recent hospitalizations, although a 54 year old male reported frequent outpatient dental visits and his 49 year old wife attends a group care centre every week.

MRSA Case 3: A middle-aged female human required hysterectomy at a small community hospital in January 2000, from which she recovered uneventfully. She was not screened for MRSA at that time. In October 2000, her 16 year old male Irish thoroughbred horse went to a large veterinary teaching hospital for surgical removal of several perirectal melanomas, requiring an overnight hospital stay. A veterinarian detected an abscess two days later, which was swabbed and grew MRSA. The abscess required incision, drainage, and a two week course of irrigation with mupirocin before completely resolving. Nasal swabs from the horse's owner subsequently grow MRSA with an identical PFGE pattern to that isolated from the horse. All veterinary staff involved in this case that was voluntarily screened were negative for MRSA.

MRSA Case 4: A female dog was involved in a car accident in May 2000 and sustained a limb fracture. The treatment modality for this fracture is unspecified; however, the animal continued to have limb pain and incomplete reduction of the fracture. The limb ultimately required amputation, and swabs from that limb grew MRSA. An elderly male in the household had previously been hospitalized for treatment of an unspecified cancer. He and the other humans in the household were subsequently screened for MRSA and found to be negative.

As the preceding four cases illustrate, identifying the exact chain of transmission for MRSA can be difficult. Because MRSA is a rare cause of infection in animals, it can often require weeks before its presence is detected. This delay in diagnosis can have obvious implications on the health of the animal. It also increases the length of time during which the animal, and its colonized human contact, are undetected MRSA carriers. Nevertheless, these cases provide some evidence suggesting that development of MRSA infections in animals indicates prior contact with humans who are at high risk of being colonized with MRSA. It should be noted that all animal MRSA isolates examined in this study exhibited PFGE patterns commonly found in human isolates.

This study suggests that, given the relative rarity of MRSA infection in animals, its isolation should necessitate the screening of close contacts including healthcare workers and household caregivers. Humans who are known MRSA carriers should use effective barrier isolation when in close contact with animals that have recent surgical wounds or are otherwise very ill. As MRSA expands its presence in the human community, there may well be a marked rise in cases of humanotic MRSA infection affecting domestic animals.

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MRSA Case	Animal	Swab Site	Antibiogram[1]								PFGE Pattern	Risk of Human Source[2]
1	9 y Bishon Frise female dog	eyelid	v	X	M	C	L	Z	E	t	K	High
2	7 y Boxer female dog	ear	v	X	m	C	L	Z	E	T	L	Low
3	17 y Irish thoroughbred male horse	buttock	v	X	m	c	l	Z	E	t	K	High
4	female dog	leg	v	X	m	c	L	Z	E	t	H	Medium

[1]Antibiogram: Each isolate was tested against the following panel of antibiotics: v=vancomycin, x=cloxacillin, m=mupirocin,c=ciprofloxacin,l=clindamycin, z=cefazolin, e=erythromycin, t= trimethoprim/sulfamethoxazole.
A small case letter denotes sensitivity, a large case letter denotes resistance, as determined by current NCCLS breakpoints.
[2]Risk of Human Source: The risk of an animal MRSA isolate being derived from a human source was estimated as follows:
High = A close human contact of the animal had multiple risk factors, such as several hospitalizations prior to the animal becoming MRSA positive, and/or the human and animal MRSA isolates are identical by PFGE pattern
Medium = A close human contact of the animal had one significant risk factor, such as a single hospitalization, prior to the animal becoming MRSA positive.
Low = There were no significant risk factors identified for close human contacts of the animal.

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Transmission of a multi-drug resistant *E. coli* in Durham Region, Ontario. A. McGeer

Third generation cephalosporin (3GC) resistance in Enterobacteriaceae is increasing worldwide. How much of this resistance is due to transmission of individual strains, plasmids or transposons, and how much is related to antibiotic pressure is unknown. Most infections and outbreaks of infection due to these organisms have occurred in the intensive care units of tertiary care hospitals. In Canada, 3GC resistance in Enterobacteriaceae remains rare. For instance, in Ontario, in 2000, fewer than 1% of *E. coli* isolates were resistant to 3GC.

In July of 2000, the infection control practitioner at the Oshawa General site of Lakeridge Health Centre identified four patients with clinical isolates of a 3GC, aminoglycoside, ciprofloxacin resistant *E. coli*. One had died of a pneumonia due to the organism; two had urinary tract infections, and one had a sputum isolate not associated with infection (this patient eventually developed pneumonia). The patients were linked not by contact within the hospital, but by residence at the same long term care facility. The subsequent, area-wide investigation has identified transmission of this strain in three acute care hospitals and 7 long term care facilities (including a retirement home and a group home for disabled children). The highest colonization rate occurred in a retirement home, in which 14/87 residents (16%) were found to be colonized when screens for rectal carriage were first performed. None of these residents had been hospitalized or had received quinolone or cephalosporin antibiotics, and none had indwelling devices. In the acute care facilities, intensive care units were not involved in transmission.

To February, 2001, 120 patients/residents have been identified as colonized or infected as part of the outbreak. Fourteen of these have been infected, and 4 deaths have been attributed to infection. In nine long term care residents, the plasmid conferring resistance has been transferred into other Enterobacteriaceae (8 different species/strains including PFGE-unrelated *E. coli*, *Enterobacter cloacae*, *K. pneumoniae*).

Carriage of the resistant organism is persistent: 36/40 (90%) of residents screened regularly for 6 months remained colonized. Despite infection control measures, some transmission in both acute and long term care is continuing.

Hospitals and long term care facilities should be aware of the possibility of rapid transmission of 3GC resistant *E. coli* in long term care facilities. Early institution of aggressive infection control measures may be needed to contain such

MicroWeb

Current CBSN susceptibility and trend data are available on the internet for easy access. The *S. pneumonie* data base can be queried directly on-line. Obtain you own password by completing the request form on the website at http://microbiology.mtsinai.on.ca. MicroWeb contains many other features of interest to laboratories, clinicians and infection control practitioners. We welcome suggestions for improving the site and making it more useful to you, our collaborators. Email us at microweb@mtsinai.on.ca

The frequency with which spontaneous *S. pneumoniae* mutants with decreased susceptibility to the FQs, can be selected when pneumococci are exposed to twice the minimal inhibitory concentration (MIC) of ciprofloxacin and levofloxacin are 1.5×10^{-7} and $>2.2 \times 10^{-6}$, respectively (5). The bacterial load of *S. pneumoniae* may be $\geq 10^8$ at the respiratory mucosal surfaces of patients with acute exacerbations of chronic bronchitis and $\geq 10^{10}$ in the lung parenchyma of patients with pneumococcal pneumonia (6;7). Therefore, a FQ treatment failure may occur either if sufficient concentrations are not achieved to inhibit or kill a susceptible strain in which a spontaneous resistance mutation has occurred, or if the infecting strain is already resistant.

Emergence of resistance in *S. pneumoniae* to the fluoroquinolones has already been described in Canada, Spain, Hong Kong and Ireland. In Canada, Chen *et al*. (8) found that the prevalence of ciprofloxacin-resistant pneumococci (MIC ≥4 μg/ml) increased from 0 percent in 1993 to 1.7% in 1997-1998 (P = 0.01). In adults, the prevalence increased from 0 percent in 1993 to 3.7% in 1998. This was associated with an increase in the consumption of fluoroquinolones. Overall, the number of fluoroquinolone prescriptions increased from 0.8 to 5.5 per 100 persons per year between 1988 and 1997 (8). Linares *et al*. (9) found that in Spain an increase of ciprofloxacin-resistant pneumococci from 0.9% in 1991-1992 to 3% in 1997-1998. Both groups of investigators found that the resistant strains were more likely to be isolated from the sputum of older patients, suggesting a possible source and reservoir for fluoroquinolone-resistant pneumococci. Ho and colleagues (10) examined the susceptibilities of 181 pneumococcal isolates from four regional laboratories in Hong Kong and found that 12% of stains had MICs to ciprofloxacin of ≥4 μg/ml. In Northern Ireland ciprofloxacin resistance was linked to penicillin resistance. Eighteen (42.9%) of 42 penicillin-resistant pneumococci were resistant to ciprofloxacin (11).

Weiss et al. (12) described an outbreak of 23F *S. pneumoniae* causing lower respiratory infection in patients on a chronic respiratory disease ward. The isolate in the first cluster of infections had an elevated MIC to ciprofloxacin of 4 μg/ml as the result of a *parC* mutation in topoisomerase IV. In the second cluster of cases the MIC had increased to 16 μg/ml as the result of an additional mutation in *gyrA*. Two patients in the first cluster (both with AECB) and three patients in the second cluster (one with hospital-acquired pneumonia and two with AECB) failed therapy with ciprofloxacin 500 mgm twice a day. Davidson et al. (13) described two patients with community acquired pneumonia due to *S. pneumoniae* that were treated as outpatients with Levofloxacin Both patients failed therapy in association with the selection of resistance to levofloxacin as the result of *parC* and *gyrA* mutations during therapy.

As the use of the respiratory FQs increases so will the prevalence of resistance. It is therefore essential that clinical laboratories perform routine in vitro susceptibility testing of all clinical isolates of *S. pneumoniae* in order to detect and report resistant strains. Although levofloxacin, moxifloxacin and gatifloxacin are valuable alternative antimicrobials for the treatment of community acquired pneumonia, we must first learn how to use them most effectively so as to prevent the emergence and dissemination of resistance and to ensure the most optimal outcome for our patients.

Streptococcal Meningitis: Straight from the Horses Mouth?

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A 49-year-female horse trainer was admitted to hospital following the rapid onset of headache, nausea, vomiting, fever, lethargy, and neck pain. She appeared toxic, was photophobic and had a decreased level of consciousness. She has a temperature of 38.0°C, a regular heart rate of 70 beats per minute, and a blood pressure of 110/70 mmHg. On exam, she had left periorbital bruising, cervical lymphadenopathy, and equal and reactive pupils. Her fundi could not be visualized. Her white blood cell count was 19.8 X 10^9/L, with a differential count of 85% neutrophils and 8% band forms. Her blood hemoglobin, platelets, electrolytes, creatinine, glucose and liver enzymes were all within normal limits. A lumbar puncture yielded cloudy cerebrospinal fluid (CSF) containing 3011 X 10^6/L neutrophils. A Gram stain revealed numerous pus cells, but no organisms. The CSF protein concentration was 1.21 g/L, and the glucose concentration was 3.3 mM, with a serum glucose concentration of 8.8 mM. *Streptococcus equi* subspecies *zooepidemicuss* (Lancefield group C) was isolated from blood and CSF cultures. She was treated empirically with IV ceftriaxone for two days, then changed to 4 mil. units IV penicillin G q4h for eight more days once the pathogen was identified. She defervesced within 24 hours and was discharged home after 11 days with some minor neurological deficits, including diplopia on looking to the left and an apparent left-sided esophoria.

Her history revealed that she cared for six horses where she worked and had two horses and a donkey of her own. All of the animals had been well with the exception of a new colt, which had just been purchased 2 months prior to her illness. The colt had signs of a respiratory tract infection including a cough and thick, yellow nasal discharge. Two weeks prior to her admission the colt kicked her in the face with his hind knee. There was no loss of consciousness or break in the skin. Two days later, she developed a sore throat, myalgia, and neck swelling, which persisted until the day of admission.

Oropharyngeal swabs were taken from her husband, her two children, her donkey and the eight horses. Swabs from her donkey and two horses at the ranch where she worked were positive for *S. zooepidemicus.* Molecular characterization of the isolates by pulsed field gel electrophoresis found that the banding patterns of the isolates from the horses were identical to the isolate from the patient but different then the isolate from the donkey .

S. zooepidemicus is a normal commensal of the skin and upper respiratory mucosa of horses. It causes wound infections and respiratory tract infections of foals and young horses as well as purulent nasal discharge and abscesses of the submandibular lymph. Group C streptococci (GCS) are rarely implicated in serious human illness. Only fourteen adult cases of GCS meningitis have been reported in the literature with a case fatality rate of 57% and the most with no obvious source of the infection.

Because few laboratories routinely determine the species of GCS, the number of human infections due to *S. zooepidemicus* is not known. Furthermore, GCS may be mistakenly identified as group A strains if only bacitracin susceptibility testing is done to differentiate group A streptococci from other B-hemolytic streptococci. This illustrates the importance of eliciting a thorough history of potential for zoonotic infections. ◆

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THANK YOU CBSN PARTICIPANTS, INVESTIGATORS, COLLABORATORS, AND SPONSORS

The Canadian Bacterial Surveillance Network continues to thrive due to your tremendous efforts. After 8 completed surveillance years, Canadian data on the epidemiology and mechanisms of resistance are being used by investigators, collaborators, and clinicians across the country to make laboratory and clinical decisions.

MicroWeb has been developed as a vehicle to share collaborative data and information. Check us out at http://microbiology.mtsinai.on.ca.

Lilyana Trpeski has moved on after 5 years of managing the CBSN office. Good luck to Lilyana and welcome to Agron Plevneshi who will be replacing her.

Thanks again to all for your continued collaboration and support.
Donald Low for Canadian Bacterial Surveillance Network