CANADIAN BACTERIAL SURVEILLANCE NETWORK

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ESBLs – "Seek and Ye Shall Find"

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An increase in ESBLs, enzymes that preferentially break down 3rd generation cephalosporins, have been detected in *E.* coli and *Klebsiella* species and have caused numerous outbreaks in American hospitals. As expected these organisms have found their way north of the border and a number of Canadian hospitals have noted an increase in resistance to 3rd generation cephalosporins such as cefotaxime, ceftriaxone and ceftazidime in their *E. coli and Klebsiella* isolates.

In *E. coli* and *Klebsiella pneumoniae*, ESBLs are usually derived from TEM or SHV β-lactamases that have mutated to expand their spectrum of activity from 2nd to 3rd generation cepalosporins (2, 3, 4). *E. coli* and *Klebsiella* acquire genes encoding these ESBLs on plasmids or transposons. TEM and SHV β-lactamases are susceptible to inactivation by inhibitors such as clavulanic acid, sulbactam and tazobactam. As well, ESBLs usually remain susceptible to cefoxitin *in vitro* (4). These features can be demonstrated using the Double Disk Test (7,9). Inactivation of the β-lactamase allows the 3rd generation β-lactam to remain active which results in the killing of the

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The Emergence of Pneumococcus with Reduced Susceptibility to the Fluoroquinolones in Canada

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Streptococcus pneumoniae is an important pathogen and the major bacterial cause of community-acquired pneumonia (CAP). Effective treatment of pneumococcal infections has until recently relied upon the use of β-lactam antibiotics, but with the emergence of antibiotic resistance, their use is now increasingly compromised. There is considerable interest in the use of alternative antimicrobials, such as the fluoroquinolones. Although older fluoroquinolones, such as ciprofloxacin, are limited in their effectiveness, newer compounds show greater promise. Such agents include levofloxacin and grepafloxacin, both of which have been recently approved for the treatment of CAP in Canada. In the near future, trovafloxacin will also be approved for treatment of CAP.

A problem associated with the use of fluoroquinolones is the selection resistance by spontaneous mutation. Several studies with pneumococci have shown that low-level resistance can result from mutations in topoisomerase IV. Increased levels of resistance can then occur following the acquisition of mutations in gyrA, which encodes the A subunit of DNA gyrase.

Recently, an efflux mechanism as a further cause of low-level resistance in pneumococci has been described. However, these descriptions are restricted to laboratory-generated mutants, and no data are available on the occurrence of this form of fluoroquinolone resistance in clinical isolates.

As part of the ongoing studies of antimicrobial resistance within the Canadian Bacterial Surveillance Network, we have been collecting data regarding the in vitro activity of ciprofloxacin against *S. pneumoniae*. In the last few years, our surveillance data indicates a

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Gram negative bacilli in the area where the antibiotic and inhibitor are combined (ie. in the area where the two diffusion zones overlap).

Some bacteria will produce ESBLs at very low levels, making them difficult to identify by automated susceptibility systems. NCCLS has had to change interpretive breakpoints and zone sizes for *E. coli* and *Klebsiella* to improve ESBL detection (6). New MicroScan panels and Vitek cards incorporating these changes are better able to detect ESBLs (5, 7). E-test manufactures ceftazidime plus ceftazidime/clavulanic acid ESBL strips that will detect ESBLs with *in vitro* activity against ceftazidime, however, some ESBLs may not be detected if only ceftazidime is used (1, 9). The Double Disk test is currently the most universally available and accessible technique capable of demonstrating the presence of ESBLs in *E. coli* and *Klebsiella*.

A lesser known cephalosporin, cefpodoxime, has been suggested by the CDC to be the most sensitive means to detect ESBLs (8) and has therefore been included in the NCCLS guidelines on testing of *E. coli* and *Klebsiella* (6). This antibiotic is available in disks which may be used to screen organisms. MicroScan has introduced a single well of 1 μg/mL cefpodoxime into their new panels (5). Growth in this well prompts a "?EBL" flag, requiring the technologist to decide whether the organism is an ESBL or not. While this antibiotic is very sensitive in the detection of resistance, it is not always specific. Some *E. coli* and *Klebsiella* elaborate higher than usual amounts of chromosomal *ampC* β-lactamase. This β-lactamase has activity against cefpodoxime but not other extended spectrum β-lactams, and is not clinically significant. Differentiating such strains from ESBL-producers is based on increased resistance to cefoxitin and the absence of -lactamase inactivator activity using the Double Disk Test. It is important that strains such as these are not mistakenly reported as ESBL.

Another mechanism causing resistance to the extended spectrum β-lactams is the production of high levels of *ampC* beta-lactamases (4). *AmpC* β-lactamase is usually chromosomally encoded in organisms such as *Citrobacter* and *Enterobacter* species. However, laboratories in the Toronto area have been isolating an increasing number of *E. coli* and *Klebsiella* resistant to 3rd generation cephalosporins with characteristics of *ampC* β-lactamases. This is not a thoroughly investigated mechanism in these genera, but may be due to the acquisition by *E. coli* and *Klebsiella* of plasmids or transposons carrying *ampD*, *ampR* and *ampC* genes originating on the chromosome of *Citrobacter* and *Enterobacter* species.

AmpC ESBL may be distinguished from TEM and SHV-type ESBLs using the Double Disk test plus cefoxitin. In contrast to the TEM and SHV ESBLs, *ampC* β-lactamase are not inactivated by clavulanic acid, sulbactam or tazobactam. In addition, organisms with high-level *ampC* production are typically resistant to cefoxitin. These characteristics, previously considered uncommon in *E. coli* and *Klebsiella*, are typical of derepressed mutants that are selected for during cephalosporin therapy in genera such as *Citrobacter* and *Enterobacter* species.

The emergence of ESBLs as potential pathogens requires a review of current laboratory practice to ensure accurate identification of these organisms and appropriate reporting of resistance to physicians who are prescribing treatment for these patients. In addition, it is important that we determine the extent of this resistance problem across Canada. This is the reason we are asking for a collection of *Klebsiella* and *E. coli* isolates to be sent from the participating surveillance sites in 1999.

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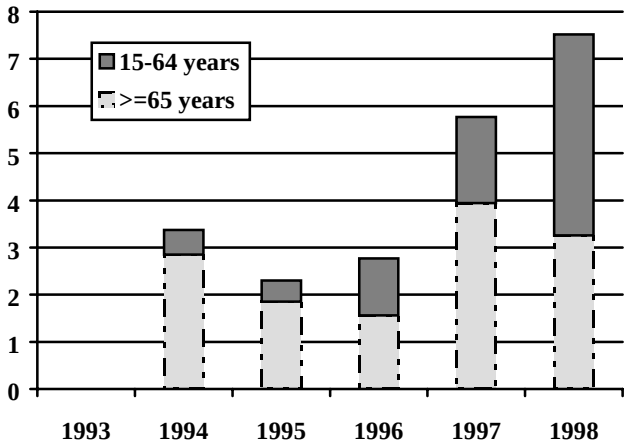
significant increase in the number of stains of pneumococci with reduced susceptibility to the fluoroquinolones as noted by the presence of an MIC to ciprofloxacin of ≥ 4 mg/L. These isolates have mutations in the *parC* and *gyr*A genes (see figure 1). The range of MICs to ciprofloxacin are from 4 to 64 mg/L, with a corresponding increase in the baseline MICs to the other newer fluoroquinolones. These strains have been exclusively isolated from patients > 15 years of age, are made up of a number of different serotypes, and have been submitted from across Canada. This will be the first prospective nation-wide study to demonstrate the emergence of pneumococci with decreased susceptibility to the fluoroquinolones. This has special significance with the recent introduction of several new fluoroquinolones which have been developed and marketed for the treatment of CAP.

These findings provide another example of the value of an ongoing network of laboratories, such as exists with the CBSN. We will be sharing this data with the medical community in the near future in order to alert physicians about the impact of inappropriate use of antibiotics and potential treatment options. In addition we will continue to monitor both the rates and the serotypes in which reduced activity is found. One very real threat is that resistance develops in a capsular serotype (e.g., 23, 9V, 6B) that is especially fit and potentially able to disseminate widely and quickly.❖

Table 1. Percentage of *Streptococcus pneumoniae* with Ciprofloxacin MIC ≥ 4



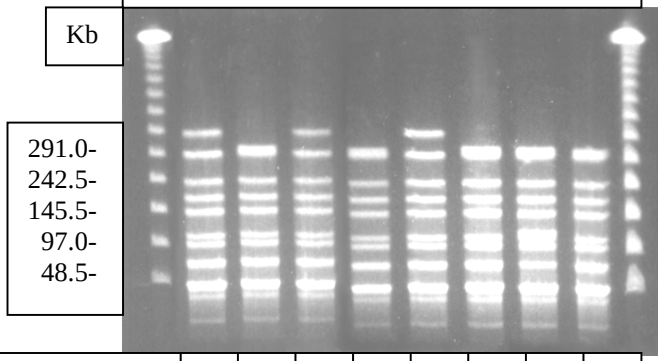
Interesting Website:
PHL Online. http://www.phlo.com/
Visit the Ontario Public Health Laboratories website. It contains lots of resource information, addresses, calendar of events and a great collection of useful web site links.

Hospital Prevention Policies and the Incidence of Early Onset Neonatal Group B Streptococcal Disease

A. Blacklock for the Toronto Bacterial Diseases Network

Invasive GBS disease is the most common infectious complication of the early neonatal period, with rates ranging from 0.5 to 4/1000 live births. The Toronto GBS study group has conducted population-based surveillance for neonatal GBS disease in metropolitan Toronto/Peel region (popn 3.1M) since 01/95. Microbiology laboratories report all GBS from sterile site cultures to the study office. Data are collected for infants born to mothers resident in the population area. Overall birth rates are obtained from hospital and census tract birth record data. Data on hospital policies and physician practice are also collected. Overall rates of early onset GBS disease were in 1.21/1000 live births in 1995, 1.08 in 1996 and 0.77 in 1997. There are 23 hospitals in which babies are delivered in the population area; data on policies were available from 20 (87%). 16/20 (80%) had GBS prevention policies. Policies were established between 1991 and 1997: 12 hospitals had developed or revised policies in 1997/8. The most common guidelines used were the Canadian Consensus Guidelines. Seven hospitals reported providing inservice training to nurses when policies were established; 10 had trained physicians, and 7 reported monitoring compliance. The existence of policies was associated with a significant reduction in rates of early onset disease: at hospitals without policies the rate of early onset disease was 1.23/1000 live births in 1995, 1.47 in 1996 and 1.21 in 1997. In contrast, rates in hospitals with policies decreased from 1.33/1000 live births in 1995 to 1.09 in 1996 and 0.77 in 1997. The provision of inservice training to nurses or physicians was not associated with greater decreases in rates; however, hospitals who reported monitoring compliance with guidelines had greater reductions than those who did not: from 1.57/1000 in 1995 to 1.00 in 1996 and 0.40 in 1997; compared to 1.39, 1.73 and 1.36 in hospitals without compliance monitoring. Incidence of disease decreased from 1.78/1000 births in 1995 to 1.05 in 1997 in hospitals with a primarily risk-based approach to prevention and from 1.17 to 0.77 in those with a screening-based approach. The existence of hospital policies, and the monitoring of compliance with these policies is associated with clinically and statistically significant reductions in rates of early onset neonatal GBS disease: these data suggest that both risk-based and screening-based approaches can affect disease incidence.❖

Figure 2. PFGE of M3 Isolates of GAS



Group A Streptococcus – The M3 Story

Dr. A. McGeer

Despite a decade of study, the reason for the re-emergence of severe invasive GAS disease in the 1980s, and for variability in illness severity remain largely unexplained. Because strains sent to references centres, and those reported in the literature as causing toxic shock and necrotizing fasciitis are more likely to be strains which possess streptococcal pyrogenic toxins (A, B or C) than average strains of GAS, the presence of these toxins may be associated with severity of illness. However, our ability to test this hypothesis has been limited.

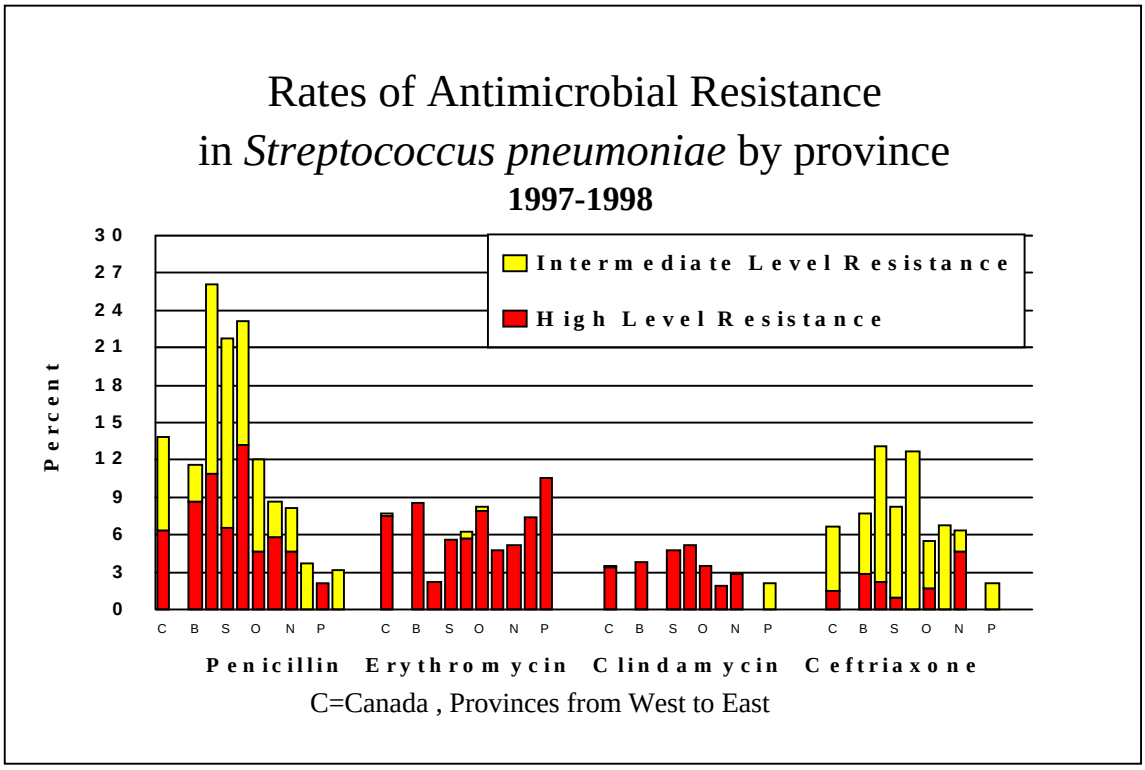
A recent analysis of infections from the Ontario GAS Study provides a valuable step forward in our understanding of virulence factors. In Ontario surveillance form 1992 to 1997, the second most common M-serotype causing infection was M3. M3 strains were responsible for 95/1127, or 8.4% of all infections. Infections due to M3 strains occurred rarely in 1992/3, then increased sharply in late 1994 and 1995, before dropping again to very low levels in 1997 and 1998.

Patients infected with M3 strains had similar primary sites of infection to other patients, with the exception that pneumonia was more common with M3 strains. Thus 44% of patients with M3 infections had a soft tissue focus, compared to 50% for others, 21% had pneumonia compared to 9% of others, and 17% had bacteremia without focus compared to 12% of others. Within the group of patients with soft tissue infections, however, patients with M3 strains were much more likely to have necrotizing fasciitis (55% vs. 20%, $P<0.001$). Patients infected with M3 isolates were also more likely than others to meet criteria for STSS (RR 2.0, 95% CI 1.2,3.2), and to die (RR 1.8, 95%CI 1.2-2.6).

50/92 (54%) M3 isolates tested contained the gene for speA, compared to 190/211 (90%) M1 isolates, and 18/564 (3%)other serotypes ($P<0.001$). All M3 isolates were clonally related when analyzed by pulsed-field gel electrophoresis (see Figure 2 below) 63% were identical to each other (type A), and an additional 27% differed by one band (type B). 40/47 type A isolates tested contained the speA gene, compared to 0/20 type B isolates. Probing of SmaI digests for speA found the gene to be located on the 300kb fragment present in type A but not type B isolate digests.

Isolates containing the speA gene were more likely to be associated with STSS (RR 1.75, 95%CI 1.2,2.5) and with death (OR 3.7, 95%CI 1.4,10), but not more likely to be associated with NF (RR 1.2, 95% CI 0.6,2.4).

Thus, within these M3 isolates, the presence of a 300 kb fragment of DNA containing the spe A gene is associated with a marked increase in severity of illness. We are currently investigating whether this fragment is a phage or part of the chromosome, and what other genes are present on it. The presence of this fragment (and the speA gene) is not, however, associated with necrotizing fasciitis, suggesting that NF and STSS have different pathogenesis.❖



Penicillin Resistance in isolates of Streptococcus pneumoniae
Numbers tested: 1994/5(1320), 1996(1044), 1997/8(1872)

	Intermediate (n(%))			Resistant (n(%))		
Province	**1994/5**	**1996**	**1997/8**	**1994/5**	**1996**	**1997/8**
BC/ALB	8(5.9)	18(16)	10(6.6)	1(0.7)	4(3.5)	14(9.3)
NWT	8(9.6)	6(10.5)	--------	3(3.6)	2(3.5)	--------
SASK	25(18)	12(14.3)	19(15.3)	3(2.2)	6(7.1)	8(6.5)
MAN	4(5.3)	12(7.1)	21(9.9)	1(1.3)	9(5.4)	28(13.2)
ONT	30(4.6)	39(8.4)	76(7.4)	15(2.3)	21(4.5)	47(4.6)
QUE	5(6.5)	2(2.1)	4(3.9)	4(5.2)	3(3.2)	6(5.8)
MAR*	5(3.2)	4(6.3)	8(2.9)	1(0.6)	1(1.6)	9(3.3)
Total	85(6.4)	93(8.9)	139(7.4)	20(2.1)	46(4.4)	119(6.4)

*Maritimes includes NB, NS, PEI, NFLD