BRIEF REPORTS

DISTRIBUTION OF STREPTOCOCCUS PNEUMONIAE SEROTYPES IN NASOPHARYNGEAL CARRIAGE AND IN INVASIVE PNEUMOCOCCAL DISEASE IN SAO PAULO, BRAZIL

Eitan N. Berezin, MD,* Maria D. Cardemilo, MD,* Leda L. Ferreira, MD,† Marcelo Otsuka, MD,‡ Maria L. Guerra, MSci,§ and Maria Cristina C. Brandileone, PhD§

Abstract: To determine whether serotypes of S. pneumoniae isolated from the nasopharynx (NP) are representative of data from patients with invasive disease, we collected NP swab specimens from children, between 3 months and 5 years and obtained data from 105 children hospitalized with invasive disease. The prevalence of penicillin nonsusceptible strains in the NP carriage and invasive disease group was 16.4% and 17%, respectively, in the first period and 42% and 45% in the second period. The serotypes 23F, 6A, 14 and 19F were the most common in the NP study and 14, 1, 5 and 6B were the most common in invasive infections.

Key Words: pneumococcus, serotypes, nasopharyngeal carriage, invasive disease

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From the *Santa Casa S. Paulo University Hospital-Pediatrics Infectious Diseases Unit; †FS. Paulo University Hospital; ‡DArci Vargas Children Hospital; and §Instituto Adolfo Lutz, S. Paulo, Brazil.

Address for correspondence: Eitan Berezin, Av Roberto Lorenz 482 S. Paulo, S. Paulo, Brazil, Cep 05611-050. E-mail: eberemin2003@yahoo.com.

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S. pneumoniae (SP) is the main etiologic agent of community-acquired pneumonia, and acute otitis media in children, especially in those younger than 5 years of age. Human nasopharyngeal (NP) carriage is the main site of acquisition of pathogenic bacteria in the respiratory tract.1–3

NP colonization with SP is a prerequisite for local and systemic disease. Several studies conducted in Israel,3 South Africa,4 Pakistan,5 Canada6 and New Guinea7 confirm that NP colonization with SP, in young children, reflects the serotype distribution and antimicrobial susceptibility of invasive disease isolates.

The main objective of this study was to determine whether serotype distribution and antimicrobial susceptibility of SP isolated from the nasopharynx of children correlated with serotype distribution and antimicrobial susceptibility of invasive isolates. The second objective was to assess the serotype distribution found in NP and invasive pneumococcal disease (IPD) isolates and calculate serotype coverage for the commercial SP heptavalent conjugated vaccine with serotypes 4, 6B, 9V, 14, 18C, 19F, 23F (7V), the investigational vaccine that contains the 10 serotypes plus 1, 5 and 7F (10V) and the other investigational vaccine that contains the 10 serotypes plus 3, 6A and 19A (13V) vaccines.

METHODS

This study was carried out at the pediatric emergency services and pediatrics units of 3 hospitals located in Sao Paulo, Brazil, in patients who had not received pneumococcal vaccine.

Children aged 3 months to 5 years with upper respiratory infections presenting to the pediatric emergency services between June 1997 and May 2001 were eligible for the study. The NP samples were obtained according with the WHO recommendations.8

For collecting the NP samples, a flexible sterile cotton-tip swab was introduced into the child’s nostril to a depth that corresponded to two-thirds of the distance between the nose and the earlobe. The swab was inoculated onto 5% sheep blood agar plate containing 5 μg/mL gentamicin, and taken to the bacteriology laboratory on the same day. Alpha-hemolytic colonies exhibiting morphologic characteristics suggestive of S. pneumoniae were isolated. Identification was confirmed by inhibition with optochin and by bile solubility.

Isolates were routinely screened for penicillin resistance with an oxacillin 1 μg disk by the Kirby-Bauer disk diffusion method. Organisms with a zone of inhibition <20 mm were confirmed as penicillin nonsusceptible (PNSP) by minimal inhibitory concentration (MIC) using the penicillin E-test and defined as susceptible, intermediate resistance or high resistance in accordance with Clinical and Laboratory Standards Institute (formally known as NCCLS) guidelines.

All SP strains were serotyped by the Quellung test using sera from the Staten Seroinstitut, Copenhagen, Denmark, at the Instituto Adolfo Lutz, Sao Paulo, Brazil.

To compare serotypes and penicillin susceptibility data we selected children aged 3 months to 5-year-old diagnosed with IPD, admitted in the same hospitals between 1996 and 2001 identified by review of microbiology records between 1996 and 2001.

Univariate analysis was performed by the χ2 test with Yates correction to confirm the significant differences between the groups of colonized and noncolonized children. P < 0.05 were considered significant. Fisher’s exact test was used when expected cell values were <5.

Proportion of coverage by the heptavalent PCV and the investigational 10 and 13v PCV vaccines were calculated by proportion of serotypes included in the vaccines of all serotypes detected in colonized and infected children.

RESULTS

NP samples were obtained from 520 children, 225 between 3 months and 2 years age and 218 between 2 and 5 years. SP was recovered from 181 children (35% of total). From this total, 109 were from children 3 months to 2 years of age and 72 from children 2 to 5 years of age.

Between 1997 and 1998, NP swabs were obtained from 440 patients. SP was isolated in 139 patients (32%). Between 2000 and 2001, NP swabs were obtained from 80 patients and SP was found in 42 patients (53%). NP colonization was found in 109 (35%) of children 3 months to 2 years of age and in 72 (32%) of those 2–5 years of age.

During the study period, 105 invasive infections were diagnosed in children less than 5 years of age. A total of 55 and 50 IPD isolates were identified in 1996–1999 and 2000–2001, respectively. In the first period, 50% of the patients were younger than 2-year-old. In the second period, 64% were younger than 2 years. The sites were pleural fluid, blood culture, CSF and articular fluid (32, 36, 34 and 3 patients, respectively). The diagnoses were pneumonia in 52 children, meningitis in 34, arthritis in 4 and bacteremia in 15.

Twenty (14%) and 18 (43%) patients were colonized by PNSP in 1997–1998 and 2000–2001, respectively. All strains exhibited intermediate susceptibility. Among the invasive infections PNSP was found in 14 of 55 (25.4%) of patients in 1997–1998 and 17 of 50 (34%) of patients in 2000–2001. All strains isolated in the first study period exhibited intermediate resistance. High-level resistance was seen in 6 strains (35% of the PNSP) in the second study period.

We identified serotypes in 162 of the 181 SP in NP isolates (120 in the first period and 42 in the second period) and 105 SP in IPD isolates (55 in the first period and 50 in the second period). Serotype distribution for NP and IPD isolates is shown on Table 1.
The most frequent serotypes obtained from the nasopharynx were 14, 6B, 23F, 6A and 19F, accounting for 52.8% of all NP isolates. The serotypes 20, 23F and 11A were found only in the first period and serotype 4 was found only in the second period.

Serotype 14 was the most frequent IPD serotype in the 2 periods accounting for 35.5% of all IPD isolates. Serotypes 14, 1, 5 and 23F accounted for 72.5% of the IPD isolates and were the 4 most frequent serotypes in both periods of the study. Serotypes 14, 1 and 5 were significantly more likely to cause invasive disease and serotypes 6A and 11A were significantly more likely to be present only as colonizers (Table 1).

In pneumonia, the most frequent serotypes were 14 (24), 1 (8) and 6B (4). In meningitis the most frequent serotypes were 23F (9), 14 (6) and 5 (6).

Serotype coverage by the 7V vaccine, by the 10V vaccine and by the 13V vaccine for NP and IPD isolates is shown in Table 1.

**DISCUSSION**

Data comparing antibiotic susceptibility between invasive and carriage *S. pneumoniae* isolates are limited. Kellner et al in Canada and Lehmann et al in Papua New Guinea studied the prevalence of reduced penicillin susceptibility in NP carriage and invasive infections found no difference between the 2 groups.

There are differences in the prevalence and rank order of serotypes between invasive and noninvasive collections and these differences vary depending on the population and geographic location. In our study, the most frequent serotypes obtained from NP were 14, 6B, 19F, 6A and 23F. The most frequent serotypes obtained from PII were 14, 1, 23F and 5. In our study, serotypes 1 and 5 were rare among NP carriage isolates, in contrast to the high prevalence of those serotypes among the isolates causing pediatric invasive disease. It has been well documented that serotypes 1 and 5 are among the most common IPD serotypes in Brazil. It has been hypothesized that, because of their virulence, serotypes 1 and 5 have a transient and fast passage through the nasopharynx before reaching the bloodstream.2 Laval et al in a report from Goiania, Brazil, reported a higher coincidence between serotypes of NP and from IPD isolates probably because in this report the rate of infections by serotype 1 was low. In our series most of infections caused by serotype 1 were pneumonia.

The differences in the prevalence and rank order of serotypes between invasive and noninvasive collections vary depending on the population and geographic location. In addition, because PNSP isolates are linked to a limited number of serotypes, any valid comparison of *S. pneumoniae* isolates from different sites must take serotypes into account.

*S. pneumoniae* is the most common cause of death worldwide due to a vaccine preventable illness. Vaccination is the best solution to this problem. Effective vaccines that include serotypes 1 and 5 would be very beneficial preventing more than 90% of IPD cases. Until these investigational vaccines become available, our study demonstrates that we can prevent the majority (66%) of IPD cases in Sao Paulo (Brazil) with the use of the 7V vaccine.

**REFERENCES**


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**TABLE 1. Serotypes (ST) Distribution of NP and IPD in the First Period (1o p) and in the Second Period (2o p), and Proportion of Coverage by 7V, 10V and 13V Vaccines**

<table>
<thead>
<tr>
<th>ST</th>
<th>NP Isolates</th>
<th>IPD Isolates</th>
<th>1o p N (%)</th>
<th>2o p N (%)</th>
<th>Total N (%)</th>
<th>1o p N (%)</th>
<th>2o p N (%)</th>
<th>Total N (%)</th>
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</tr>
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<td>1 (2.4)</td>
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<td>7 (13.6)</td>
<td>6 (12)</td>
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<td>5</td>
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<td>6 (12)</td>
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<tr>
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<td>8 (4.8)</td>
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<td>4 (8)</td>
<td>13 (12.4)</td>
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<td>55</td>
<td>50</td>
<td>105</td>
<td>—</td>
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<td>7V</td>
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<td>30 (71)</td>
<td>95 (55.4)</td>
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<td>32 (64)</td>
<td>68 (64)</td>
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<tr>
<td>10V</td>
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<td>33 (78)</td>
<td>103 (62)</td>
<td>48 (69)</td>
<td>46 (90)</td>
<td>94 (89)</td>
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<tr>
<td>13V</td>
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<td>37 (88)</td>
<td>129 (78)</td>
<td>51 (92)</td>
<td>47 (94)</td>
<td>98 (93)</td>
<td>NS</td>
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<td></td>
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</table>

*P is comparing total serotypes of NP and IPD isolates.
NS indicates nonsignificant.
Others—NP-Nontypeable-4; 19A-3; 22, 23A, 33 one each IP-7F: 3; 3 and 33 one each.


TRANSMISSION OF INFLUENZA A IN FAMILIES

Stephen S. S. Teo, MBBS,* Joanna S. Ellis, PhD,† Celia Aitken, MRCPath,‡ and Robert Booy, MD*§

Abstract: We conducted a prospective, nested study using nucleotide sequencing to examine influenza positive respiratory samples in families for genetic homology in the hemagglutinin and neuraminidase genes. Influenza A H3N2 viruses from 3 families had identical, family specific, HA1 nucleotide sequences. Sequences among these families were genetically heterogeneous. A 4th family was distinguished by sequencing of the influenza neuraminidase gene.

Key Words: influenza, transmission, families, sequences

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From the *Centre for Child Health, Barts and the London, Queen Mary’s School of Medicine and Dentistry, Queen Mary, University of London, UK; †Respiratory Virus Unit, Enteric, Respiratory and Neurological Virus Laboratory, Centre for Infections, Health Protection Agency, Colindale, UK; and ‡Virology Clinical Group, Barts and the London NHS Trust, London, UK.

Celia Aitken’s current address is Regional Virus Laboratory, Glasgow, Scotland.

Robert Booy’s current address is The National Centre for Immunisation Research and Surveillance, The Children’s Hospital at Westmead, Sydney, Australia.

Address for correspondence: Dr. Stephen Teo, Centre for Child Health, Barts and the London School of Medicine and Dentistry, Queen Mary College, University of London, 4 Newark Street, London E1 2AX, United Kingdom.

E-mail: teosss@yahoo.com.

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Influenza transmission was studied 20 years ago using viral culture and serology. Schoolchildren were major introducers of influenza into families, but younger children were also important.1,2 The difficulty in distinguishing influenza infection acquired at home from that acquired in the community was acknowledged.2

Today, more young children spend time in out-of-home care and may have overtaken older school age children as the primary source of influenza transmission in communities.3 Polymerase chain reaction (PCR) based assay enables nucleotide sequencing of different strains.4 Nonsynonymous nucleotide substitutions in the hemagglutinin (HA) gene can result in amino acid changes in the antigenic sites in the HA1 domain.5 The rate of these substitutions is higher in HA1 than in HA2 or in the neuraminidase (NA) gene.6,8

We studied a population of children younger than 5 years and their parents to ascertain whether a child-to-parent mode of transmission would be supported by nucleotide sequencing of influenza viruses.

METHODS

We recruited children younger than 5 years who were enrolled in a burden of influenza study in east London, United Kingdom, during the 2002–2003 influenza season.9 Ethics approval was granted by the North East London Research Ethics Committee. Influenza-positive children were identified by immunofluorescence (IF) assay of nasopharyngeal aspirate samples. Inclusion criteria for parents were fluency in English or Bangladeshi, ability to provide informed consent, absence of a contraindication to the taking of a nasal wash sample, and the development of symptoms within 14 days after the onset of symptoms in their child.

A nasal wash was obtained from symptomatic parents. One aliquot was examined by IF for influenza A and B, respiratory syncytial virus, adenovirus and parainfluenza 1, 2 and 3. The other aliquot was stored at 4°C before transfer to storage at −70°C and subsequently transported to the Health Protection Agency, Colindale.

For comparison, field viruses representative of influenza viruses circulating during 2002–2003 were also analyzed. Samples were first analyzed by multiplex nested reverse transcription-PCR (RT-PCR) to type and subtype the HA gene.10 A multiplex PCR for the subtyping of the NA gene was performed using nested primer sets.11 The nucleotide sequence of the HAI1 and HAI2 domains of the HA gene of influenza A viruses, and the coding region of the NA gene, were determined by amplification from cDNA, using a high-fidelity DNA polymerase (Pfu Ultra, Stratagene, Netherlands). Both strands of purified amplicons were sequenced with appropriate PCR primers.11,12 Phylogenetic analysis of sequences was performed using Mega3.13 Nucleotide sequences were submitted to the Los Alamos Influenza Sequence Database14 and assigned unique accession numbers ISDN119037–ISDN119066.

RESULTS

Influenza Diagnosis by IF or PCR. We recruited 9 children and 10 parents from 7 families (Table 1). Eight nasopharyngeal aspirate samples from these children were influenza A positive by IF and PCR examination. Influenza A H3N2 was detected in 7 samples and A H1 in one child. Families F1 and F2 were related and the children were in close contact. All other families were separate in time and space. We obtained nasal washes from 6 of 8 parents who became symptomatic after their children. Although nasal wash samples from all parents were negative by the IF assay for respiratory viruses, subsequent multiplex RT-PCR assays detected influenza A H3N2 in 4 of these samples.

Nucleotide Sequence Analysis of the HAI1 Domain of the HA Gene of Influenza A H3N2 Viruses. We obtained parent–child paired viruses from 3 families (F2, F4 and F6). In all 3 families the HAI1 sequences of the influenza A H3N2 viruses analyzed from the family members were identical within that family (Fig. 1). Sequences from 2 of the families (F2 and F4), and from the index cases from 2 families (F1 and F3), were closely related to A/Panama/2007/99 (Fig. 1).

The HAI1 nucleotide sequences of viruses from families F1, F2, F3 were identical and differed by 3 nucleotides from HAI1 sequences from family F4. Of these 3 nucleotide differences, 2 were nonsynonymous and resulted in the amino acid substitutions V112 and E280K. The HAI1 sequences from the other family (F6) were most closely related to the H3N2 variant, A/Fujian/411/2002. HAI1 sequences from viruses in this family F6 differed by 29–32 nucleotides from those analyzed from families F1, F2, F3 and F4, and by 14–16 amino acid differences.

Eight of the 11 field isolates analyzed were genetically similar to A/Panama/2007/99-like viruses (Fig. 1). Of these, 2 viruses had identical HAI1 sequences (A/England/449/2002 and A/England/459/
and 6 viruses had HA1 sequences differing by 2–17 nucleotides, and 1–10 amino acid substitutions. Three other field strains were genetically similar to A/Fujian/411/2002-like viruses (A/England/448/2002, A/England/356/2003 and A/England/408/2003), and differed from each other by 7–21 nucleotides, encoding 3–7 amino acid substitutions.

Nucleotide Sequence Analysis of the HA2 Domain of the HA Gene, and the NA Gene of Influenza A H3N2 Viruses. No nucleotide differences were identified between the HA2 sequences of viruses from families F1, F2 and F3, or F4, although the HA2 sequences of viruses from these families (F1, F2, F3 and F4) differed from the HA2 sequences from family F6 by 13 nucleotide and 4 amino acid substitutions.

Sequence analysis revealed that the NA genes of viruses analyzed from F1, F2, F3 and F4 were genetically similar to the NA from A/Panama/2007/99-like viruses, whereas the NA of viruses from F6 were A/Fujian/411/2002-like. NA sequences of viruses analyzed were identical within each family, but differed among families F1, F2, F3 and F4 by 1–3 nucleotides and from the viruses detected in family F6 by 47–50 nucleotides. The NA sequence of the virus from the index case in family F3 was distinguished from those of viruses from family F1 and F2, by a single nonsynonymous substitution at nucleotide 205, encoding the amino acid substitution I62M.

DISCUSSION

We demonstrated family specific genetic homology in the HA1 domain of the HA gene of virus strains detected in 3 families with young children (F2, F4 and F6). The HA1 nucleotide sequences differed among these families by at least 3 nucleotides. In each family, a parent became ill 1–7 days after the index subject. This is consistent with a child-to-parent mode of transmission. We also found genetic homology in respiratory samples from child contacts who became ill within 1–8 days of each other, consistent with child–child transmission as well. The specificity of HA1 nucleotide sequencing in inferring transmission is higher than that of a clinical diagnosis, serology, culture, IF or PCR analysis of viral isolates. Our results are consistent with the results of Gubareva and colleagues15 who demonstrated identical HA1 nucleotide sequences recovered from families infected with influenza. Our study was performed with genetically different circulating viruses and in children aged <5 years who were specifically excluded from this previous study.

The HA1 sequences of viruses detected in 3 families (F1, F2 and F3) were identical, although families F1 and F2 were

### TABLE 1. Influenza A Viruses Recovered From Cases, 2003

<table>
<thead>
<tr>
<th>Family</th>
<th>No. of Family Members</th>
<th>Index/Contact</th>
<th>Status of Influenza A Positive Subjects</th>
<th>Subject Age (yr)</th>
<th>Interval*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Recruited</td>
<td>Ill</td>
<td>Tested</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td>3</td>
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<td>1</td>
<td>Index</td>
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</tr>
<tr>
<td>F2</td>
<td>4</td>
<td>4</td>
<td>4</td>
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</tr>
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<td>Contact</td>
<td>21</td>
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</table>

N/A: not applicable.
*Interval in days between onset of symptoms in the index case and symptoms in the contact.
†Became symptomatic 8 days after index from F2.
‡One parent tested.
§Both parents tested.

**FIGURE 1.** Phylogenetic analysis of the HA1 sequences of influenza A H3 viruses using Mega3 with a neighbor-joining algorithm. The lengths of the horizontal lines are proportional to the nucleotide differences (%) between sequences as indicated by the bar. I = index; C = contact; F = family.

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related. Analysis of the HA2 sequences was not able to discriminate between viruses from F1, F2 and F3. When the analysis was extended to the NA gene of the viruses from families F1, F2 and F3, a single nucleotide difference distinguished the viruses from F1 and F2, from the virus detected in F3. The observed substitutions between the HA and NA sequences from families F1, F2, F3 and F4 were present in both strands using forward and reverse sequencing primers. The NA mutation that differentiated F3 from F1 and F2 was also confirmed by repeat sequencing from the original sample material. This study was limited by the small number of cases because of the low activity influenza season. Our use of IF to identify index cases missed children who were influenza-positive only on subsequent PCR testing. IF was used due to the shorter turn-around time compared with block-based reverse transcription PCR assay.9 Despite the small sample size the isolates within families were more homologous than viruses isolated from sporadic cases in the same season.

The application of nucleotide sequencing in surveillance of influenza viruses should assist in the prediction and investigation of influenza transmission in clusters other than families such as day care or entire communities, and thus help us understand the impact of different vaccination and antiviral use strategies.

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REFERENCES


CHANGE IN PNEUMOCOCCAL SUSCEPTIBILITY TO AZITHROMYCIN DURING TREATMENT FOR ACUTE OTITIS MEDIA

Philip Toltzis, MD, Michael Dul, PhD, and Jeffrey Blumer, PhD, MD

Abstract: Authorities have suggested restriction of azithromycin use as a principal strategy to contain the spread of azithromycin-nonsusceptible Streptococcus pneumoniae (ANSP). In 83 children persistently colonized by pneumococcus during and after treatment of acute otitis media, 17 acquired a new strain, 9 of which were less susceptible to azithromycin than the original isolate. New appearance of ANSP was documented after both β-lactam and azithromycin exposure. ANSP is likely to disseminate even with significant reduction of azithromycin use unless other antibiotic use is decreased as well.

Key Words: antibiotic resistance, Streptococcus pneumoniae, azithromycin, acute otitis media

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From the Department of Pediatrics, Rainbow Babies and Children’s Hospital, Case Western Reserve University School of Medicine, Cleveland, OH. Supported by grant HD 31323-05 from the National Institutes of Health for the Pediatric Pharmacology Research Unit, and by grants from Roche and Pfizer Pharmaceuticals.

Address for correspondence: Philip Toltzis, MD, Rainbow Babies and Children’s Hospital, 11100 Euclid Avenue, Cleveland, OH 44106. E-mail: ptx2@case.edu.

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Macrolide antibiotics have long been safe and effective choices for treatment of community-acquired respiratory-tract infections. During the past 15 years, however, resistance to the macrolides in Streptococcus pneumoniae and other respiratory-tract pathogens has increased coincident with their increased use. This observation has led some experts to recommend reducing macrolide use as a principal measure to limit the spread of macrolide resistance.1–3 This strategy presupposes that the acquisition of macrolide-resistant pneumococci is associated specifically or disproportionately with antecedent macrolide exposure. To test this supposition, we studied longitudinally a group of children treated for acute otitis media (AOM) and measured the frequency with which organisms of differing susceptibilities to azithromycin and penicillin appeared in the nasopharynx during and shortly after therapy.

MATERIALS AND METHODS

The samples in the current study were a subset of those derived from a larger program examining the effects of antibiotics on the microbial colonization patterns of ambulatory children.4 In the larger study, children aged 3 months to 7 years with a diagnosis of AOM were recruited from 16 suburban pediatric practices in the Cleveland, OH, metropolitan area. Enrolled children were randomized to receive amoxicillin (80 mg/kg/d in 2 divided doses), cefpro-
zil (50 mg/kg/d in 2 divided doses), azithromycin (10 mg/kg on the first day, followed by 5 mg/kg on each of the next 4 days), or a single intramuscular dose of ceftriaxone (50 mg/kg). Nasopharyngeal swab specimens were obtained at entry, and then on days 3–5, 10–14, and 28–30 after initiation of therapy, and were processed for the presence of S. pneumoniae as previously described.4

Frozen stocks of pneumococcal isolates, archived during approximately the first-third and last-third of the study, constituted the organisms analyzed for the current study. The antibiotic susceptibility to azithromycin was determined by microdilution assay and to penicillin by E-test. Organisms were categorized as azithromycin-nonsusceptible S. pneumoniae (ANSP) and penicillin-nonsusceptible S. pneumoniae (PNSP), if the minimal inhibitory concentrations (MIC) of the antibiotic were ≥1 μg/mL and ≥0.1 μg/mL, respectively, consistent with guidelines suggested by the National Committee for Clinical Laboratory Standards (recently renamed the Clinical and Laboratory Standards Institute). Organisms isolated from the same child were categorized as having “different susceptibilities” if the MICs to an antibiotic differed by >4-fold.

Serotyping was determined by the Quellung reaction, using antisera obtained from the Statens Serum Institute, Copenhagen. Clonal relationships were established by pulsed-field gel electrophoresis (PFGE) using routine techniques.5 The mechanism of resistance to macrolide antibiotics was determined by testing the organisms for the presence of the efflux pump mefE and the ribosomal methylase ermB by PCR, using previously described methods.6

RESULTS

The larger study examining the differential effects of antibiotics on colonization by pneumococcus commenced in November 1999 and extended through 3 winter seasons. Three hundred forty-eight subjects, of 1009 enrolled, were colonized in the nasopharynx at least 1 of the 4 study visits. Frozen stocks of pneumococcal isolates were archived from 221 colonized children. Subjects from whom frozen specimens were available did not differ from those in whom isolates were not available with regard to age, day-care attendance, history of AOM in the past 30 days or the past 6 months, or treatment assignment (data not shown, all P > 0.21).

Eighty-seven of these 221 subjects had S. pneumoniae isolated from their nasopharynx on more than 1 study visit. In 3 instances, 1 of the organisms failed to grow from frozen stocks, and in 1, PFGE analysis was precluded because of autolysis of DNA. Organisms from the remaining 83 children were analyzed.

Sixty-six of the 83 children (79.5%) carried identical PFGE types through the period of observation, and 17 (20.5%) carried more than 1 PFGE type. Among the 66 subjects with repeated isolates of the same PFGE type, there was no instance in which there was a change in susceptibility pattern to either azithromycin or penicillin over successive study visits. In 20 of these 66 instances, the organism was susceptible to both penicillin and azithromycin, and in 38 the organism was nonsusceptible to both.

In the remaining 17 subjects, pneumococcal isolates successively cultured from the same child demonstrated discordant PFGE types. In all circumstances where the PFGE type changed, the serotype also changed. In 14 of 17 instances (Table 1), susceptibility to penicillin, azithromycin, or both differed from the original isolate, whereas in the remaining 3 subjects, the isolates retained the same susceptibility pattern despite being derived from different PFGE types. In 9 of the 14 instances in which the susceptibility phenotype changed, the organism isolated later in the observation period was equally or more resistant to both penicillin and azithromycin than the isolate earlier (subjects 1–9, Table 1). In 5 subjects, however, the later-isolated organism was more susceptible to penicillin or azithromycin or both (subjects 10–14, Table 1). The appearance of a new PFGE type occurred in children receiving all 4 study treatments. Among subjects acquiring a PFGE type with an MIC to azithromycin higher than that measured in their original isolate, 3 were assigned to azithromycin, 2 to cefprozil, and 3 to ceftriaxone. New PFGE types that were nonsusceptible to azithromycin expressed a wide range of MICs and were positive for mef, for erm, and for both determinants.

DISCUSSION

The current report indicates that, in most instances, acquisition of ANSP is the result of the appearance of a new strain rather than acquisition of resistance by an old one. These observations suggest that, similar to PNSP, the mechanism of acquisition is eradication of susceptible organisms from the nasopharynx allowing

### TABLE 1. Phenotypic Changes in 17 Subjects Colonized With a New PFGE Type

<table>
<thead>
<tr>
<th>Subject Number</th>
<th>Visits*</th>
<th>Penicillin MIC*</th>
<th>Azithromycin MIC*</th>
<th>Treatment*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pneumococci with different MICs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>V0 → V1 → V3</td>
<td>0.016 → 0.064 → 0.190</td>
<td>0.05 → 0.05 → 256(E)</td>
<td>AZT</td>
</tr>
<tr>
<td>2</td>
<td>V0 → V1</td>
<td>0.02 → 0.064</td>
<td>0.12 → 256(E)</td>
<td>AZT</td>
</tr>
<tr>
<td>3</td>
<td>V0 → V3</td>
<td>0.012 → 1.5</td>
<td>0.12 → 256(EM)</td>
<td>CFZ</td>
</tr>
<tr>
<td>4</td>
<td>V0 → V1</td>
<td>0.047 → 2.0</td>
<td>0.06 → 256(EM)</td>
<td>CTX</td>
</tr>
<tr>
<td>5</td>
<td>V1 → V2</td>
<td>2 → 2</td>
<td>0.125 → 16(M)</td>
<td>AZT</td>
</tr>
<tr>
<td>6</td>
<td>V0 → V1 → V3</td>
<td>0.023 → 0.125 → 0.190</td>
<td>0.05 → 256(E) → 256(E)</td>
<td>CTX</td>
</tr>
<tr>
<td>7</td>
<td>V1 → V3</td>
<td>0.125 → 4</td>
<td>0.05 → 256(EM)</td>
<td>CTX</td>
</tr>
<tr>
<td>8</td>
<td>V1 → V3</td>
<td>0.015 → 1</td>
<td>0.12 → 4(M)</td>
<td>CFZ</td>
</tr>
<tr>
<td>9</td>
<td>V0 → V2</td>
<td>0.016 → 0.380</td>
<td>0.05 → 0.05</td>
<td>AZT</td>
</tr>
<tr>
<td>10</td>
<td>V0 → V1</td>
<td>8 → 0.016</td>
<td>&gt;256(E) → 256(EM)</td>
<td>CTX</td>
</tr>
<tr>
<td>11</td>
<td>V0 → V3</td>
<td>1 → 0.023</td>
<td>2 → 0.05</td>
<td>CTX</td>
</tr>
<tr>
<td>12</td>
<td>V2 → V3</td>
<td>0.015 → 1</td>
<td>8(M) → 0.05</td>
<td>AMX</td>
</tr>
<tr>
<td>13</td>
<td>V0 → V3</td>
<td>2 → 0.5</td>
<td>&gt;256(EM) → 0.12</td>
<td>CTX</td>
</tr>
<tr>
<td>14</td>
<td>V0 → V3</td>
<td>2 → 0.125</td>
<td>&gt;256(EM) → 0.06</td>
<td>CTX</td>
</tr>
<tr>
<td>Pneumococci with same MICs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>V0 → V3</td>
<td>0.016 → 0.016</td>
<td>0.06 → 0.06</td>
<td>AMX</td>
</tr>
<tr>
<td>16</td>
<td>V0 → V1</td>
<td>0.023 → 0.016</td>
<td>0.05 → 0.05</td>
<td>CFZ</td>
</tr>
<tr>
<td>17</td>
<td>V0 → V3</td>
<td>0.016 → 0.016</td>
<td>0.05 → 0.05</td>
<td>AMX</td>
</tr>
</tbody>
</table>

*V0 indicates visit 0 at baseline; V1, visit 1 at 3–5 d; V2, visit 2 at 10–14 d; V3, visit 3 at 28–32 d; MIC, minimal inhibitory concentration; AMX, amoxicillin; CFZ, cefprozil; CTX, ceftriaxone; AZT, azithromycin.

1For isolates resistant to azithromycin, the mechanism of resistance is denoted in parenthesis: (M), mef; (E), erm; (EM), both mef and erm.
either new colonization or overgrowth of a pre-existing resistant subpopulation. Because eradication of susceptible pneumococci is common to many oral antibiotic regimens, it can be anticipated that community spread of ANSP will occur after exposures to several different antibiotic classes including but not limited to macrolides.

This preliminary analysis was performed because the molecular basis for β-lactam and macrolide resistance is different, prompting us to question whether the dynamics of acquisition of PNSP and ANSP colonization might be different as well. β-Lactam resistance among S. pneumoniae is mediated by the expression of chromosomally-encoded abnormal penicillin binding proteins. The molecular events leading to the generation of these proteins are rare. Consequently, the epidemiology of penicillin-resistant pneumococci is one characterized by the horizontal transmission of a small number of predominant strains. In most children, colonization by 1 pneumococcal serotype at least partially inhibits colonization by a second. The acquisition of PNSP by individuals receiving antibiotics is primarily the result of destabilization of the nasopharyngeal ecology, particularly eradication of penicillin-susceptible pneumococci, which in turn allows either overgrowth of a cocolonizing β-lactam-resistant clone or colonization by a new organism through horizontal transmission, presumably from a close contact.

By contrast, most macrolide resistance in pneumococcus is mediated either through expression of an erm ribosomal methylase or one of the mef family of efflux pumps. Unlike β-lactam resistance, both of these determinants potentially may be newly expressed in a given clone under antibiotic pressure. Expression of many of the erm and mef genes is inducible in the presence of macrolide, and at least some of these genes are harbored on transmissible elements, which may be exchanged among cocolonizing bacteria in the presence of antibiotic. These observations raise the possibility that macrolide/azalide resistance in pneumococcus arises principally from originally susceptible clones that then emerge resistant after antibiotic exposure. This possibility would strongly support restriction of azithromycin use as a strategy to contain the expansion of ANSP in the community.

Our data, however, did not support this latter event, but rather suggested that acquisition of PNSP and ANSP both occurred as a result of the appearance of a new PFGE type after destabilization of the nasopharyngeal ecology caused by antibiotic exposure. Our data did not allow us to determine the relative propensity of the different treatment regimens to promote colonization by ANSP. The frequency of coincident resistance to penicillin and azithromycin seen in this and other studies, however, suggests that repopulation by ANSP strains regularly occurs after β-lactam exposure, and by PNSP after azithromycin exposure. Consistent with this speculation, studies conducted in Finland and Greece have documented an association between the regional frequency of macrolide-resistant pneumococci and local consumption of both macrolides and β-lactam drugs.

The recommendation to limit azithromycin use as the principal strategy to decrease the spread of ANSP is based largely on studies correlating increased regional consumption of the drug with the population incidence of azithromycin resistance, a design that is inherently inferential. It is probable that ANSP originally emerged as a result of macrolide and azalide exposure. With the established endemic presence of these strains in the community, however, the current study raises the possibility that they may disseminate even with significant reduction of azithromycin use unless attention is directed toward decreasing the use of other antibiotics as well.

REFERENCES

Efficacious interventions to prevent mother-to-child transmission (MTCT) of human immunodeficiency virus type 1 (HIV-1) have been developed: antiretroviral (ARV) prophylaxis, cesarean section before labor and before ruptured membranes (ECS), and complete avoidance of breast-feeding.1 Such interventions have been recommended as part of guidelines for the management of HIV-1-infected women in various countries.2– 6 As a consequence, there has been a substantial reduction in MTCT of HIV-1 in many countries, including those in Latin America and in the Caribbean,2– 6 and elimination of MTCT of HIV-1 is now considered a reasonable goal in certain settings.7 In previous analyses of data from the National Institute of Child Health and Human Development (NICHD) International Site Development Initiative (NISDI) Perinatal Study, a prospective cohort study of HIV-1-infected women and their infants in Latin America and the Caribbean, 99% of women received ARVs during pregnancy (over 80% received 3-drug ARV combination regimens), 38% of the women delivered by ECS, and none of the infants was breast-fed. Two factors were suggestive of an association with an increased risk of MTCT of HIV-1: mothers not receiving ARVs at the time of enrollment into the study and infant low birth weight. Our objectives in this study were to describe cases of MTCT of HIV-1 occurring in this cohort, and to evaluate whether opportunities for the prevention of transmission were missed.

### MATERIALS AND METHODS

The NISDI Perinatal Protocol is a prospective cohort study conducted at multiple clinical sites in Latin America and the Caribbean where ARVs for prevention of MTCT of HIV-1 and alternatives to breast milk (eg, infant formula) are available.7 Enrollment into the NISDI Perinatal Protocol began in September 2002, and is ongoing. Maternal study visits are conducted during pregnancy, at labor/delivery, at hospital discharge after delivery, and postpartum at 6–12 weeks and at 6 months. During these study visits, the women are interviewed, a physical examination is conducted, and laboratory studies are obtained (except at the labor/delivery and the 6 months postpartum visits). Physical examination of the infants is conducted, and laboratory studies obtained, at birth and at 6–12 weeks and 6 months after birth.

The study population for this article consists of HIV-1-infected infants born to women enrolled in the NISDI Perinatal Protocol as of March 2006. Infants were categorized as HIV-1-infected if they had any 2 of the following (obtained from separate specimens): positive HIV-1 culture, positive HIV-1 DNA PCR assay, positive neutralizable HIV-1 p24 antigen assay, or quantitative HIV-1 RNA \( \geq 1000 \) copies/mL. In the absence of breast-feeding, transmission was categorized as having occurred in utero or intrapartum according to 2 definitions. According to the first definition, transmission was presumed to have occurred in utero if one or more virologic assays were positive within the first 2 days of life.

### TABLE 1. Characteristics of 8 HIV-1-Infected Infants

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Cases</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5 (Twin A)</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in days at first positive HIV-1 diagnostic test</td>
<td>In utero</td>
<td>1</td>
<td>44 (2)</td>
<td>90 (1)</td>
<td>64 (7)</td>
<td>7</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV-1 diagnostic test results (in copies/mL if plasma HIV-1 RNA concentration; noted as positive or negative if qualitative DNA PCR result)</td>
<td>Hospital discharge after birth</td>
<td>219,000</td>
<td>1894</td>
<td>Undetectable</td>
<td>Undetectable</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
<td>622</td>
</tr>
<tr>
<td>Hospital discharge after birth 6–12 wk visit</td>
<td>984,000</td>
<td>330,000</td>
<td>2.8 Million</td>
<td>2.5 Million</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>235,810 and 1.8 million (2 specimens)</td>
<td></td>
</tr>
<tr>
<td>Hospital discharge after birth 6 mo visit (if age of first HIV-1 diagnostic test different from age at first positive test, then age at first test in parentheses)</td>
<td>330,000</td>
<td>286,000</td>
<td>1.7 Million</td>
<td>1.3 Million</td>
<td>Positive and 3723</td>
<td>Positive and 12,152 (2 specimens)</td>
<td>30,189</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estimated timing of MTCT of HIV-1</td>
<td>Definition 1 (see text)</td>
<td>In utero</td>
<td>In utero</td>
<td>Intrapartum</td>
<td>Intrapartum</td>
<td>Intrapartum</td>
<td>Intrapartum</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>Definition 2 (see text)</td>
<td>Birth weight (kg)</td>
<td>2.6</td>
<td>3.4</td>
<td>2.0</td>
<td>2.9</td>
<td>2.3</td>
<td>1.9</td>
<td>1.1</td>
<td>2.6</td>
</tr>
<tr>
<td>Estimated gestational age at birth (completed wk)</td>
<td>37</td>
<td>41</td>
<td>39</td>
<td>39</td>
<td>36</td>
<td>34</td>
<td>30</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Infant antiretroviral prophylaxis regimen</td>
<td>ZDV</td>
<td>ZDV</td>
<td>ZDV</td>
<td>ZDV</td>
<td>ZDV</td>
<td>ZDV</td>
<td>ZDV</td>
<td>ZDV</td>
<td></td>
</tr>
</tbody>
</table>

†If age of first HIV-1 diagnostic test is different from age at first positive test, the age at first test is in parentheses.
Presumed intrapartum transmission was defined as negative virologic assays during the first week, with evidence of HIV-1 infection between 7 and 90 days of age. According to the second definition, a child with the first positive HIV-1 assay at ≤7 days of age was classified as having in utero infection. Intrapartum infection was defined as a negative HIV-1 assay at ≥7 days of age, with a first positive assay >7 days of age. Missed opportunities for prevention were cases of MTCT where it is possible that different medical (ARV prophylaxis or treatment; complete avoidance of breastfeeding) and/or surgical (ECS) management would have prevented MTCT of HIV-1.

RESULTS

Between September 2002 and March 2006, 988 HIV-1-infected women were enrolled, 950 (96.2%) of whom acquired HIV-1 infection through heterosexual contact. Of these 988 women, 911 had delivered live born infants and the others either were still pregnant or had other pregnancy outcomes. Of the live born infants, 820 had completed the 6-month visit, 8 of whom were HIV-1-infected [transmission rate, 0.98% (95% CI: 0.45–1.96%)]. These 8 infants were born in Argentina (4 infants from 2 hospitals in Buenos Aires) or Brazil (1 infant from Rio de Janeiro, 2 infants from Ribeirao Preto, and 1 infant from Porto Alegre), and none was breast-fed. Other characteristics of the infants and their mothers are shown in Tables 1 and 2. One woman reported alcohol use during pregnancy (case 2), whereas 3 women were smokers (cases 1, 6, 7). None of the women reported any other substance abuse during pregnancy.

Of the 2 infants with presumed in utero transmission according to both definitions, one (case 1) was born to an asymptomatic smoker with a high plasma viral load (VL) copies/mL and a low CD4 count (CD4) at baseline who received a 3-drug ARV regimen beginning at 24 weeks gestation, but who did not show major improvement in VL or CD4 count. The other infant (case 2) was born to a woman who initiated a 3-drug ARV regimen at 30 weeks gestation. Both of these cases were considered missed opportunities.

TABLE 2. Characteristics of Mothers of 8 HIV-1-Infected Infants

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Gestational age at enrollment (wk)</td>
<td>21</td>
</tr>
<tr>
<td>Baseline clinical classification</td>
<td>A</td>
</tr>
<tr>
<td>Baseline viral load (copies/mL)</td>
<td>52,000</td>
</tr>
<tr>
<td>Baseline CD4 count (cells/mm³)</td>
<td>185</td>
</tr>
<tr>
<td>Obstetrical estimate of gestational age at initiation of receipt of antiretroviral drugs (wk)</td>
<td>24</td>
</tr>
<tr>
<td>Actual duration of receipt of antiretroviral drugs during pregnancy (wk)</td>
<td>10</td>
</tr>
<tr>
<td>Antepartum antiretroviral regimen(s)</td>
<td>ZDV/3TC/NFV</td>
</tr>
<tr>
<td>Intrapartum ARV regimen(s)</td>
<td>ZDV</td>
</tr>
<tr>
<td>Mode of delivery</td>
<td>Vaginal</td>
</tr>
<tr>
<td>Duration of ruptured membranes (h)</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Episiotomy</td>
<td>Yes</td>
</tr>
<tr>
<td>Laceration</td>
<td>No</td>
</tr>
<tr>
<td>Number of days between delivery and hospital discharge</td>
<td>2</td>
</tr>
<tr>
<td>Clinical classification at hospital discharge following delivery</td>
<td>A</td>
</tr>
<tr>
<td>Viral load at hospital discharge after delivery (copies/mL)</td>
<td>28,000</td>
</tr>
<tr>
<td>CD4 count at hospital discharge after delivery (cells/mm³)</td>
<td>207</td>
</tr>
</tbody>
</table>

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(case 1: because the woman did not show significant improvement in VL or CD4 despite receipt of a combination ARV regimen for several weeks during pregnancy, and it is possible that more intensive ARV management could have resulted in an uninfected infant; case 2: because ARV prophylaxis was only initiated at 30 weeks gestation, and it is possible that transmission could have been prevented if ARV prophylaxis had been initiated earlier in pregnancy).

Of the 4 infants with presumed intrapartum transmission according to both definitions, 1 infant (case 3) was a low birth weight but term infant whose mother, although asymptomatic and with a low VL, had a decrease in her CD4 despite 2 combination ARV regimens during pregnancy. The next infant (case 4) was born to a woman who delivered vaginally but who had a VL of 11,151 at hospital discharge after delivery. Another infant (case 5) was born to a woman with extremely high VLs, CDC Class C disease, and with duration of ruptured membranes of 6 hours. Of note, this infant’s twin (Twin B) was uninfected. The delivery was by cesarean section, with the primary indication being prevention of HIV-1 transmission. A third infant (case 6) was delivered by ECS (primary indication: prevention of HIV-1 transmission) to a mother who received only 2 weeks of zidovudine prophylaxis initiated at 28 weeks gestation (despite an extremely elevated VL at enrollment). The VL remained above 1000 copies/mL at the time of hospital discharge after delivery. These 3 cases (cases 4 – 6) were considered missed opportunities [case 4: Because of the vaginal delivery, assuming a VL obtained prior to delivery would be similar to the VL at hospital discharge; ECS (if acceptable to the mother) might have prevented transmission; case 5: For the same rationale as for case 1; and case 6: Because only zidovudine was administered as prophylaxis beginning at 28 weeks, despite the very high VL at enrollment].

Of the 2 infants with unknown timing of transmission according to one definition and in utero or intrapartum transmission according to the second definition, one (case 7) was born after prolonged duration of ruptured membranes (>24 hours) and the mother had received only 2 weeks of zidovudine prophylaxis (beginning at 27 weeks) before delivery. The other infant (case 8) was born to a woman with a long duration of ruptured membranes (15 hours) and with a perineal laceration.

**DISCUSSION**

Numerous risk factors for MTCT of HIV have been identified or are under investigation. General categories of risk factors include: the amount of virus to which the child is exposed (eg, higher maternal plasma VL), the duration of such exposure (eg, vaginal delivery, longer duration of ruptured membranes), factors facilitating the transfer of virus from mother to child (eg, chorioamnionitis), and the child’s susceptibility to infection (eg, preterm birth and low birth weight). Although many different interventions to prevent MTCT of HIV-1 have been and are being investigated, efficacy has been demonstrated to date for only ARV prophylaxis, ECS, and avoidance of breast-feeding. In addition to these interventions, observational data strongly suggest that receipt of combination ARV regimens is associated with very low rates of transmission. Our observed transmission rate is very low — consistent with data from other studies of HIV-1-infected women who have access to ARVs not only for prevention of MTCT, but also for treatment of their own HIV-1 disease, who are receiving care at centers that provide cesarean section for prevention of MTCT as indicated, and who have access to replacement feeding for their infants such that breast-feeding can be completely avoided.

MTCT of HIV-1 can occur in utero, around the time of labor and delivery, or postnatally through breast-feeding. None of the 8 HIV-1-infected infants breast-fed, and thus transmissions occurred in utero or intrapartum. Risk factors for in utero or intrapartum MTCT of HIV-1 were present in all of the 8 mother-infant pairs [including advanced maternal HIV-1 disease (high maternal VL, low maternal CD4 count, advanced maternal clinical HIV-1 disease), low birth weight, and/or preterm birth]. However, in 5 of these 8 cases (cases 1, 2, 4 – 6) there appeared to be missed opportunities for prevention of MTCT, ie, instances in which MTCT of HIV-1 might have been prevented with different medical (ARV prophylaxis or treatment) or surgical (ECS) management. Our results serve to emphasize the importance of ongoing training and education regarding prevention of MTCT of HIV-1, as well as continued research regarding mechanisms of and interventions to prevent such transmission.

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ACUTE HUMAN IMMUNODEFICIENCY VIRUS INFECTION IN A BREAST-FED INFANT IN NEW YORK CITY

Ouzama Nicholson, MD, MS, David E. Michalik, DO, Sameer Patel, MD, Philip LaRussa, MD, and Natalie Neu, MD

Abstract: Acute human immunodeficiency virus (HIV) infection in a breast-fed infant is a rare diagnosis in developed countries. We present a six-month old girl with postnatally acquired HIV infection complicated by Pneumocystis jiroveci pneumonia, cytomegalovirus pneumonia and encephalopathy. Her mother had tested negative for HIV during pregnancy. Children infected by mothers during an acute seroconversion may have more rapid disease progression.

Key Words: acute HIV infection, mother-to-child transmission, breast-feeding, cytomegalovirus, Pneumocystis carinii pneumonia

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From the Department of Pediatrics, Columbia University and New York Presbyterian Hospital, New York, NY.

Address correspondence to: Ouzama Nicholson, MD, MS, Columbia University, Division of Pediatric Infectious Diseases, 622 W 168th Street, PH Building 4th Floor, Room 465, New York, NY 10032; E-mail: onicholson1@partners.org.

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With the advent of regimens to prevent mother-to-child transmission of human immunodeficiency virus (HIV)-1 infection and highly active antiretroviral therapy, mother-to-child transmission of HIV has declined significantly in the United States.6,7 Transmission of HIV infection from mother-to-child may occur in utero, at the time of delivery or postnatally through breast-feeding.5,9 In the United States, women known to be HIV-infected are advised against breast-feeding. However, this recommendation can only be followed if women are aware of their HIV status. Pregnant women may have higher rates of HIV seroconversion than other women.10,12 Hence, testing done early in pregnancy may not identify all women at risk for transmitting HIV to their infants, and repeat testing has been recommended.12,13 Furthermore, women infected postnatally may continue to breast-feed and unknowingly infect their infants.

We present a case of postnatally acquired HIV infection in a 6-month-old breast-fed infant presenting with fever and pancytopenia. Her mother tested negative for HIV in both the second and third trimesters of pregnancy and the child’s New York State newborn screen was negative for HIV antibodies. Despite multiple previous negative tests, the mother tested positive for HIV after the child’s diagnosis. Clinicians should have a high index of suspicion for HIV infection in children presenting with signs and symptoms of acquired immunodeficiency syndrome (AIDS), despite negative testing during pregnancy and the newborn period.

CASE REPORT

A 6-month-old girl was admitted to our hospital for further evaluation of pancytopenia after initially presenting to an outside hospital with a 2-day history of fever, rhinorrhea and bilateral eyelid swelling. She was a full-term, vaginally delivered, breast-fed infant. Her past medical history was remarkable for viral gastroenteritis at 3 months of age and immune thrombocytopenia purpura at 5 months of age, both requiring hospital admission. Her initial physical examination was remarkable only for white plaques on the buccal mucosa. Laboratory evaluation was remarkable for anemia, (hematocrit 23%), thrombocytopenia (platelets 117,000/mL) and elevated serum transaminase values: aspartate aminotransferase (AST) 436 units/liter, alanine aminotransferase (ALT) 111 units/liter.

She was transferred to our hospital for bone marrow biopsy, which was normal. The fever persisted for 1 week and she was treated empirically with piperacillin-tazobactam for 10 days despite negative cultures. On the 12th hospital day she developed a rash and evidence of hemolysis with an acute drop in her hematocrit from 27% to 21%, and a positive direct Coombs test. Her serum lactose dehydrogenase concentration rose to 4611 units/liter and AST to 1435 units/liter. Two days later she developed increased irritability and fever. A lumbar puncture was performed and revealed a total protein of 483 mg/dL, glucose of 58 mg/dL, 2 white blood cells/mm3, and 0 red blood cells/mm3. She was treated empirically with ceftriaxone, acyclovir and lipid complex amphotericin B. Thirty-six hours later she appeared obtunded and a repeat lumbar puncture showed improved parameters, with total protein of 158 mg/dL and no increased leukocytosis. By the following day, her mental status improved remarkably. She was afebrile with a resolving hepatitis. Consent for HIV testing was obtained. HIV enzyme linked immunosorbent assay was positive, Western blot was indeterminate and HIV RNA viral load was >750,000 copies/mL. Trimethoprim-sulfamethoxazole was initiated several days later for Pneumocystis jiroveci pneumonia prophylaxis.

Within a week of her HIV diagnosis she developed tachypnea and worsening respiratory distress requiring continuous positive airway pressure. A bronchoscopy showed evidence of Pneumocystis jiroveci on silver stain and intranuclear inclusions consistent with cytomegalovirus (CMV). The dose of trimethoprim-sulfamethoxazole was increased and corticosteroid therapy was added with significant improvement. Antiretroviral therapy with lopinavir-ritonavir, stavudine and lamivudine was initiated. She was discharged to home to complete a 21-day course of trimethoprim-sulfamethoxazole with steroid taper and to continue antiretroviral therapy.

However, within a week of discharge she presented with fever, tachypnea, lymphocytosis and a multilobar consolidation on chest tomography. Despite broad-spectrum antimicrobial and antifungal therapy, her respiratory status declined eventually requiring high frequency ventilation. A viral culture from her tracheal aspirate grew CMV thought to reflect viral shedding. At that time, her CD4+ count had dropped from 2095 cells/µL (56%) at her initial diagnosis to 407 (54%) and her HIV viral load had dropped from >750,000 to 29,851 copies/mL. Plasma CMV polymerase chain reaction (PCR) was >100,000 copies/mL. Induction therapy with ganciclovir followed by CMV immune globulin was initiated for CMV pneumonitis. The CMV PCR decreased to 1880 copies/mL after 2 weeks of
induction, and became undetectable after initiating maintenance therapy. She was transitioned to conventional ventilation 1 month after being hospitalized and was discharged a few weeks thereafter.

Despite 6 months of highly active antiretroviral therapy, the patient’s HIV RNA viral load remains at detectable levels. Her outpatient course has been complicated by an episode of varicella infection, renal tubular acidosis and developmental delay.

The infant’s mother reported testing negative for HIV infection on 2 separate occasions during pregnancy. Review of the child’s New York State newborn screen showed that routine testing for HIV-1 specific antibodies was negative. At our request, the sample was later screened for HIV by DNA PCR, which was also negative.

At the time of diagnosis, the child had a positive enzyme linked immunosorbent assay, indeterminate Western blot and high viral load, all suggestive of acute infection. On further questioning, the child’s mother reported a 2-week illness comprised of fever, sore throat and swollen glands when the child was approximately 2 months of age. Maternal HIV serologies were positive during the child’s hospitalization. Given negative serologies during pregnancy and at the time of delivery, we suspect that this illness represented an acute retroviral syndrome in the mother and that the child was infected during an acute maternal seroconversion.

**DISCUSSION**

This case represents a rare report of acute HIV infection in a breast-fed infant in a developed country. It also reflects the rare occurrence of postnatal transmission of HIV infection during an acute maternal seroconversion. Since the advent of the use of zidovudine and more potent treatment regimens for HIV-infected pregnant women,1–7 new infections among infants have become rare in the United States.6,7 Since 1995, the Centers for Disease Control and Prevention has recommended that HIV testing be routinely offered to all pregnant women in the United States.13 In 1999 New York State implemented testing for HIV-1 antibodies as a routine part of the state newborn screen. In our case, testing for HIV antibodies both early and late in pregnancy and at the time of delivery was negative, suggesting that there was no maternal seroconversion by the time of delivery. Furthermore, DNA PCR testing on the sample provided for the newborn screen was also negative suggesting that the child was unlikely to have been infected in utero.

We hypothesize that this child’s mother was infected some time in the postnatal period and that the child was infected at or close to the time of the mother’s acute seroconversion. Our patient’s prolonged viral syndrome, including fever, pancytopenia, hepatitis, encephalitis, and hemolysis may all be explained by an acute retroviral syndrome.15 Whereas both acute HIV infection in adults15–17 and the natural history of children infected in utero or perinatally has been well described,18–21 fewer reports exist of children undergoing acute seroconversion postnatally as a result of breast-feeding.22 One report examining clinical signs and symptoms associated with acute seroconversion among breast-feeding African children found that 3 clinical signs/symptoms, including a mononucleosis-like syndrome, dermatitis and generalized lymphadenopathy were independently associated with cases of acute HIV seroconversion as compared with controls.22

Despite a 6-month course of HAART, our patient’s viral load remains detectable. Genotype testing has revealed that she has HIV-1 subtype AG, which is consistent with the parents’ country of origin. Data suggest that AG recombinants may have up to a 2.5-fold greater change in IC50 or IC90, depending on the protease inhibitor used, when compared with the non-Ghanaian subtype B protease.23 However, given that our patient has done clinically well, we have not opted to change her regimen. Furthermore, there is limited pharmacokinetic information on other potent regimens in this age range and limited knowledge on optimal antiretroviral therapy for non-B subtypes to support a change in therapy.

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Chronic granulomatous disease (CGD) is a genetic primary immunodeficiency disease of abnormal phagocytic function. The underlying defect is an inability of phagocytes to reduce molecular oxygen and create the reactive oxygen metabolites necessary for efficient intracellular killing activity. Catalase positive organisms cause the majority of infections in CGD because only these pathogens survive inside CGD phagosomes. Catalase positive organisms include species.1-4 Fungal osteomyelitis comprises 22% of CGD patients with osteomyelitis,1 and sacral osteomyelitis with Inonotus tropicalis is a very rare presentation.

CASE REPORT

Our patient was diagnosed at age 1 year with an X-linked CGD after recurrent pneumonias, gastroenteritis and granuloma formation. He was given prophylactic trimethoprim–sulfamethoxazole at diagnosis, recombinant interferon γ at 8 years and itraconazole prophylactic therapy after treatment of an Aspergillus fumigatus osteomyelitis at 10 years. He later developed severe hemolytic anemia and vertebral osteomyelitis, which was treated with amphotericin B lipid complex and granulocyte infusions. The details of his early course are discussed elsewhere.3 At 21 years of age, he developed a left paraspinal abscess (Fig. 1A, Online only; see www.pidj.com for Fig. 1A–G) while receiving itraconazole prophylaxis. An unidentified golden brown mold grew from tissue obtained during surgical drainage. It was initially considered a contaminant because it did not produce diagnostic structures on a variety of media and culture conditions suitable for known human pathogens. The abscess resolved with surgical drainage and antibiotic therapy but no specific antifungal therapy.

At 22 years, he developed a tender mass on his left lower back with no overlying erythema or neurologic compromise. He was afebrile with an erythrocyte sedimentation rate of 21 mm/h, had normal bilateral hip plain radiographs, and a CT scan of his chest, abdomen and pelvis with contrast revealed a 3 cm × 7 cm × 1.5 cm area of increased enhancement in the lumbosacral joint and the junction of the left lumbar spine. There was a mixed sclerotic and lytic process within the sacrum. At the sacral foramen, a small air bubble was noted. A MRI (Fig. 1B) showed extensive abnormal marrow signal in the sacrum with disease extending into the sacral foramina and intracanalicular fat at S1-S2. A left paraspinal lesion was noted at L5.

Biopsies performed with ultrasound guidance of the right hemisacrum, left paraspinal abscess and bone marrow were obtained and grew the same golden brown mold that had been isolated 9 months prior. The biopsies pathology showed necrotizing granulomatous inflammation and septic fungal organisms (Fig. 1C, D). Molecular studies unequivocally identified the etiologic agent as Inonotus tropicalis. In vitro antifungal MIC data for amphotericin B, itraconazole, voriconazole, posaconazole and caspofungin at 48 hours was 0.25, 0.25, 0.5, 1, and 2 μg/L, respectively. Details of these studies are published elsewhere.5

He was treated with amphotericin B lipid complex 200 mg IV daily and voriconazole 200 mg orally twice daily. He was discharged from the hospital on voriconazole monotherapy after 2 weeks. An MRI 3 months later suggested a new lesion in the left paraspinal muscles at T4–5, an enlarging collection in the left paraspinal muscles T6–7 and a fluid collection extending caudally in the left paraspinal/gluteal region (Fig. 1E).

He was given amphotericin B lipid complex 200 mg IV thrice weekly, caspofungin 50 mg IV daily and voriconazole was continued. His fungal infection was stable on this regimen until he was admitted 6 months later for pneumonia. MRI of his thoracolumbar spine showed increased enhancement in the subcutaneous soft tissues overlying the left paraspinal muscle mass at L5/S1 and a 1-cm rim enhancing collection in the left posterior paraspinal muscle mass from T6 to T8. Bone scan was normal. The left paraspinal lesion was incised and drained with isolation of Inonotus species.

After 3 weeks of noncompliance with IV caspofungin, he had enlargement and tenderness of the left parasacral lesion with overall progression on MRI with loculated abscesses in the left paraspinal muscles. Biopsy revealed epithelioid granulomas with scattered multinucleated giant cells and septate, partly degrading hyphae. He continued triple antifungal therapy but developed the abscesses extended along the left upper buttocok and the right piriformis on MRI. With compliance to medications, the parasacral abscess was culture negative for 4 months, but with noncompliance, subsequent cultures have been positive for I. tropicalis. Susceptibility studies have shown decreased sensitivity only to caspofungin with an increased 48 hour MIC (4 μg/L). He has had intermittent drainage, swelling and pain in his left hip. Three years after triple antifungal therapy was initiated, there is still residual inflammation on MRI. (Fig. 1F, G).

DISCUSSION

The case of I. tropicalis infection described here is unique because of the insidious onset of a slowly destructive sacral osteo-
myelitis associated with soft tissue abscesses. Aspergillus is the second most common pathogen in osteomyelitis in CGD after Serratia species. When Aspergillus affects vertebral bodies, the clinical sequela in CGD patients involves paraparesis or paraplegia. Although Aspergillus is the most prevalent fungal infection in these patients, other fungi have also been reported. These include Paecilomyces variotii, Paecilomyces lilacinus, Acremonium strictum, Sarcinosporon inkin, Exophiala dermatitidis, Pseudallescheria boydii and Chrysosporium zonatum. Of these non-Aspergillus fungal infections in CGD, Paecilomyces species are most prevalent, comprising about 44% of non-Aspergillus fungal infections and 8% of total CGD osteomyelitis cases. The sites of involvement of Paecilomyces in CGD have included lung, bone, abdominal wall and soft tissue in a heel.

I. tropicalis mycosis was an unexpected finding in our CGD patient. I. tropicalis is a filamentous fungal species in the family Hymenochaetaceae and the class Basidiomycetes. It is ubiquitous and one of the largest genera of wood destroying fungi. The spectrum of documented infections reported for the basidiomycetes, namely Schizophyllum commune and Coprinus species, include endocarditis, menigitis, sinusitis, ulcerative lesions of the hard palate, fungal ball in the lung, allergic bronchopulmonary conditions, bronchial mucoid impaction and chronic respiratory disease. Although filamentous basidiomycetes fungi are being increasingly recognized, their definitive identification is problematic because many isolates do not initially produce diagnostic structures in culture.

Inonotus (also known as Phellinus) species have been studied for their potential antioxidant, antiangiogenic, tumoricidal and immunostimulatory effects. Inonotus (Phellinus) rimosus extract was able to restore cisplatin-depleted renal catalase function, suggesting that the Inonotus species can produce catalase.

Osteomyelitis in CGD tends to occur in the thoracic vertebral, ribs, and metatarsals. Fungal or mycobacterial infection tends to result from direct spread of the infection from an adjacent focus of soft tissue infection. Historically, treatment of fungal infection in CGD has included antifungal chemotherapy, surgical debridement of infected tissue and granulocyte transfusions, with the first 2 modalities typically required to eradicate fungal bone infection. We used only chemotherapy in our patient. Susceptibility studies have shown that this Inonotus species has remained susceptible to amphotericin B, itraconazole, voriconazole and posaconazole but that some resistance to caspofungin has developed over time. Since the clinical relevance of antifungal resistance and tolerance in vitro has not been well demonstrated in the literature, and because of the nature of the underlying immunodeficiency in our patient, multidrug therapy seemed prudent. Granulocyte transfusions in CGD are supported by the principle that a small number of normal phagocytes can be able to compensate for the oxidative defect in CGD phagocytes, but the efficacy of such treatments is unknown. This therapy was precluded in our patient who has the McLeod phenotype due to prior sensitization.

Surgical debridement was not a good option in this case because of the extensive mixed lytic-sclerotic destruction of the sacrum and tissue surrounding the sacral foramina. To date, the patient has had no neurologic sequelae from the infection, but the risk of extensive or even limited surgical debridement with possible sacral neurologic impairment exceeds the potential benefit of decreased organism burden. In one series, limited intrasosseal debridement effectively eradicated 5 of the 11 infections in autosomal CGD patients, but only 2 of 8 infections in X-linked CGD patients.

The prognosis for our patient is guarded. The Inonotus osteomyelitis has slightly improved on his current antifungal regimen but has not been eradicated. Bone marrow transplantation in CGD patients with extensive fungal disease can be very dangerous although successful cases have been reported. The timing of transplantation is of critical importance. If transplantation is delayed to adolescence or later, the chances of invasive fungal infections, inflammatory complications and graft-versus-host disease increase.

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LUMBOPEITONIAL SHUNT INFECTION DUE TO CHRYSEOBACTERIUM INDOLOGENES

Hossam Al-Tatari, MD, Basim I. Asmar, MD, and Jocelyn Y. Ang, MD

Abstract: Chryseobacterium indologenes central nervous system infection has not been reported. We present a case of lumbopeitonal shunt infection caused by C. indologenes successfully treated
with trimethoprim–sulfamethoxazole and rifampin in a pediatric patient. Forty-three additional cases of C. indologenes non-central nervous system infections reported in the English medical literature were reviewed. Risk factors for C. indologenes infections include underlying medical illnesses, underlying immunocompromising conditions and presence of indwelling intravascular devices.

**Key Words:** Chryseobacterium indologenes, lumboperitoneal shunt, central nervous system, infection

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From the Division of Infectious Diseases, Carman and Ann Adams Department of Pediatrics, Children's Hospital of Michigan, Wayne State University, Detroit, Michigan.

Address for correspondence: Jocelyn Y. Ang, MD, Division of Infectious Diseases, Carman and Ann Adams Department of Pediatrics, Children's Hospital of Michigan, Wayne State University, 3901 Beaubien Blvd, Detroit, MI 48201. E-mail: jang@med.wayne.edu.

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Infections caused by Chryseobacterium (previously Flavobacterium) indologenes are rare but have been reported as a cause of indwelling device-associated infections, ventilator-associated pneumonia, bacteremia, pyonephritis, peritonitis, biliary tract infection, surgical and burn wound infections. Central nervous system (CNS) infection caused by C. indologenes has not been previously reported.

**CASE REPORT**

A 13-year-old boy with congenital hydrocephalus and an existing lumboperitoneal (LP) shunt presented with complaints of fever, headache and abdominal pain for 2 days. The LP shunt had been revised several times because of malfunctions and Staphylococcus aureus and Staphylococcus epidermidis infections. He had history of multiple drug allergies, including penicillin and cefaclor. Lumboperitoneal shunt malfunction secondary to infection was suspected.

The LP shunt was externalized, cerebrospinal fluid (CSF) samples were sent for bacterial cultures and intravenous (IV) vancomycin treatment was started. The computed tomography (CT) scan of the brain and ultrasound examination of the abdomen was unremarkable. Subsequently, CSF culture grew methicillin susceptible Staphylococcus aureus (MSSA). Serial CSF cultures obtained every other day during vancomycin therapy were sterile and the child improved clinically.

Headaches and fever spikes returned on day 7 of vancomycin therapy. Repeat CSF culture grew a Gram-negative, non-lactose fermenting, oxidase-positive bacillus. Intravenous cefazidime was empirically started. Antimicrobial susceptibility testing of the organism using a minimal inhibitory concentration (MIC) method was reviewed resistance to cefazolin (MIC >16 μg/mL), cefepime (>16 μg/mL), ceftobutin (>32 μg/mL), cefazidime (>16 μg/mL), ceftriaxone (>32 μg/mL), cefuroxime (>16 μg/mL), imipenem (>8 μg/mL), meropenem (>8 μg/mL), gentamicin (>8 μg/mL), tobramycin (>8 μg/mL) and vancomycin (MIC >16 μg/mL). The isolate was susceptible to ciprofloxacin (<1 μg/mL), piperacillin/tazobactam (<8 μg/mL), rifampin (<1 μg/mL) and trimethoprim–sulfamethoxazole (TMP-SMX) (<2/38 μg/mL). Based on the susceptibility report, vancomycin and cefazidime were discontinued and IV TMP-SMX treatment was started. During TMP-SMX therapy, headaches persisted with occasional fever spikes. Because culture of the CSF on day 5 of therapy grew the same organism, IV rifampin treatment was added, but 2 days later the CSF culture remained positive. For this reason, the LP shunt was removed. The patient's condition improved after the procedure, the headache and fevers resolved and the CSF culture obtained 1 day after the procedure was sterile. The organism was identified as C. indologenes based on being a Gram-negative, non-lactose fermenting, oxidase positive bacillus. On trypsinase soy agar with 5% sheep's blood, the colonies were deep yellow. Biochemical reactions on this strain were positive for tryptophane, urease, esculin hydrolysis, gelatin, acid in maltose, and citrate. The organism grew at 25°C and 35°C, but not at 42°C.

Trimethoprim–sulfamethoxazole and rifampin treatment was continued for 14 days after the LP shunt removal. Subsequent CSF cultures remained sterile and a new LP shunt was placed. The patient continued to do well up to the time of discharge and during follow-up visit 2 weeks later. He had no recurrence of C. indologenes infection during the subsequent 2 years.

**DISCUSSION**

Chryseobacterium species are aerobic, oxidase positive, non-motile Gram-negative rods that produce a distinct yellow to orange pigment. Six species of Chryseobacterium are more commonly isolated from clinical specimens: C. meningosepticum, C. odoratum, C. multivorans, C. breve and group Iib Chryseobacterium species, which includes C. indologenes and C. gleum. Chryseobacterium are not part of the human flora. They are found in many environmental habitats including plants, foodstuff, soil and fresh and marine water. They have been recovered from indwelling vascular catheters, vials, sink traps, feeding tubes and disinfectants found in hospitals.

Chryseobacterium infections in humans are usually nosocomially and are mostly seen in immunosuppressed patients. C. meningosepticum is the most common species that causes human disease. C. indologenes, is an uncommon human pathogen. Reported infections caused by C. indologenes include ventilator-associated pneumonia, indwelling device-associated infection, bacteremia, pyonephritis, peritonitis, biliary tract infection, ocular infections and surgical and burn wound infections (Table 1). Most of the C. indologenes infections in humans have been reported from Taiwan (38 cases). Other reported cases have been from Australia, United States and Europe. Two other cases of C. indologenes (corneal abscess and pneumonia in an immunocompromised host) from Europe were reported in Spanish and are not reviewed here.

Hsu et al from Taiwan reported 36 patients, with C. indologenes infections from a single institution over a 3-year period. Common underlying conditions included neoplastic disease, diabetes, burn wounds, uremia, biliary tract stones, congenital heart disease, myocardial infarction, thrombocytopenic purpura and chronic pancreatitis. Most patients (53%) had indwelling devices (intravascular catheters, endotracheal tubes), 17 patients (47%) had bacteremia and 4 had polymicrobial bacteremia. Mortality was 14% in part a result of inappropriate antimicrobial treatment.

Lin et al from Taiwan, reported a case of C. indologenes bacteremia in a 35-year-old bone marrow transplant recipient who had a Hickman catheter. Treatment was successful with piperacillin–tazobactam without removing the Hickman catheter.

A 47-year-old patient from Taiwan with corneal ulcer and keratitis due to C. indologenes developed corneal perforation and severe vision loss despite cefazidime topical treatment and corneal flap surgery.

Kienzle et al from Australia reported 2 cases of C. indologenes infection in patients with extensive burn injury who had received first aid remedy with untreated water. C. indologenes was recovered from debrided skin of both, tracheal aspirate of one and vascular catheter of the other. Both responded to surgical debridement and IV vancomycin, rifampin and ciprofloxacin.

Nulen et al reported a 38-year-old woman receiving chemotherapy who had recurrent C. indologenes bacteremia. She was
treated with IV piperacillin–tazobactam, followed by IV pefloxacin and removal of the intravascular device.

Christakis et al,10 from Greece, reported a 54-year-old male receiving chemotherapy with C. indologenes bacteremia. He recovered after receiving 10 days of IV piperacillin–tazobactam.

Green et al11 from the United States reported a case of a 77-year-old man with leg cellulites, at the site of curettage of a superficial squamous-cell carcinoma, and bacteremia caused by C. indologenes. He swam daily in his private swimming pool. He was cured after receiving IV piperacillin–tazobactam and gentamicin for 4 days, followed by oral levofloxacin for 14 days.

We believe that our patient is the first case of a CNS shunt infection caused by C. indologenes. It is unclear how he acquired the infection. However, he most likely acquired it nosocomially since the infection developed during his hospital stay at a time that he was receiving vancomycin therapy for LP shunt infection caused by MSSA.

In general, Chryseobacteria are resistant to most antimicrobial agents prescribed for Gram-negative infections.2 They also produce beta-lactamase and are resistant to beta lactam antibiotics. Chryseobacterium is the only Gram-negative bacillus for which vancomycin has been effective,3 but it can have varying antimicrobial susceptibilities to vancomycin, rifampin and ciprofloxacin.5,9 Ciprofloxacin has also been effective against Chryseobacterium.5,9 Chryseobacterium indologenes is uniformly resistant to extended spectrum penicillin, first and second generation cephalosporins and aztreonam.3 There is varied susceptibility to clindamycin, erythromycin, tetracycline, piperacillin, aminoglycosides, third generation cephalosporins, imipenem and quinolones.3,5,14 Most C. indologenes are susceptible to TMP-SMX and rifampin and the combination therapy has been used for treatment of persisting C. indologenes infections.3

### TABLE 1. Reported Cases of Chryseobacterium indologenes Infections*

<table>
<thead>
<tr>
<th>Country</th>
<th>Year</th>
<th>Author</th>
<th>No. of Cases</th>
<th>Patients' Age (yr)</th>
<th>Type of Infection</th>
<th>Treatment</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taiwan</td>
<td>1997</td>
<td>Hsueh PR et al6</td>
<td>36</td>
<td>1–85</td>
<td>Biliary tract infection (7)</td>
<td>Not detailed</td>
<td>10 patients developed sepsis complications: Five died (2 peritonitis, 2 biliary tract infection, 1 bacteremic pneumonia). Five survived. Remaining 26 patients recovered</td>
</tr>
<tr>
<td>Taiwan</td>
<td>2003</td>
<td>Lin et al7</td>
<td>1</td>
<td>35</td>
<td>Bacteremia in a bone marrow transplant recipient with chronic graft-versus-host disease</td>
<td>14 d treatment with pipracillin–tazobactam without removing the Hickman catheter</td>
<td>Recovered</td>
</tr>
<tr>
<td>Taiwan</td>
<td>1997</td>
<td>Lu and Chan8</td>
<td>1</td>
<td>47</td>
<td>Keratitis</td>
<td>4 wk of ceftazidime eye drops and corneal flap surgery</td>
<td>Recovered with significant vision loss</td>
</tr>
<tr>
<td>Australia</td>
<td>2001</td>
<td>Kienzle et al9</td>
<td>2</td>
<td>21 and 41</td>
<td>Burn wounds</td>
<td>Silvadene topical therapy, surgical debridement and IV vancomycin, rifampin and ciprofloxacin. Treatment duration not specified</td>
<td>Both recovered</td>
</tr>
<tr>
<td>Europe</td>
<td>2001</td>
<td>Nulen et al4</td>
<td>1</td>
<td>38</td>
<td>Recurrent bacteremia associated with implanted intravascular device</td>
<td>First episode treated with pipercillin–tazobactam for 10 d</td>
<td>Recovered</td>
</tr>
<tr>
<td>Europe</td>
<td>2005</td>
<td>Christakis et al10</td>
<td>1</td>
<td>54</td>
<td>Bacteremia in a patient with solid tumor</td>
<td>Hickman catheter removed. IV pipercillin–tazobactam for 10 d</td>
<td>Recovered</td>
</tr>
<tr>
<td>United States</td>
<td>2001</td>
<td>Green and Nolen11</td>
<td>1</td>
<td>77</td>
<td>Cellulitis with secondary bacteremia</td>
<td>Piperacillin–tazobactam and gentamicin, followed by levofloxacin for 14 d</td>
<td>Recovered</td>
</tr>
<tr>
<td>United States</td>
<td>2006</td>
<td>This study</td>
<td>1</td>
<td>13</td>
<td>Lumboperitoneal shunt infection</td>
<td>Ceftazidime, then TMP/SMX. Rifampin was added and the shunt was removed</td>
<td>Recovered</td>
</tr>
</tbody>
</table>

*In English Medical literature.
REFERENCES


