PCR in CSF and one showed positive antigens in CSF (Table 1). For 5 patients, blood culture was positive for *N. meningitidis*; all showed positive CSF culture. The serogroup distribution was serogroup B for 18 cases (78%), C for 3 cases (13%) and others for 2 cases (9%). Subsets of 11 isolates (48%) were genotyped by using MLST. Five ecs were found, all from serogroup B, with the following distribution: one isolate was not assigned to any known cc (UA) (9%), 2 cc 162 (18%), 2 cc 213 (18%), 3 cc 41/44 (27%) and 3 cc 32 (27%). Phenotypes (serotypes/serosubtypes) were obtained for 16 cases (65%) from the 23 cases; one serogroup C phenotype (2a: P1.5,2) and 15 serogroup B phenotypes: 3 NT:P1,4, 2 1:P1,14 and one each of the following: 14:non-subtypable (NST); 14:P1.7,16; 1:NST; 1:P1.14,16; 1:P1,4; N:CST; NT:P1,12; NT:P1,16; NT:P1,7; NT:P1,9. The minimum inhibitory concentration (MIC) was tested for cefotaxime, amoxicillin and penicillin G for 17 strains. All tested isolates were susceptible to cefotaxime. MICS ranged from 0.002 to 0.032 mg/L. The MIC was ≤0.01 mg/L for 94% of isolated strains and ≤0.032 mg/L for 100%. For amoxicillin, MICS ranged from 0.047 to 0.38 mg/L. The MIC was <0.25 mg/L for 88% of isolates. The other isolates (12%) showed intermediate susceptibility to amoxicillin. For penicillin G, MICS ranged from 0.032 to 0.25 mg/L. The MIC was <0.12 mg/L for 88% of isolates. The other isolates (12%) showed intermediate susceptibility to penicillin G.

Two patients died (9%, girls, cared for at home, who showed late-onset meningitis at days 10 and 23, respectively).

**DISCUSSION**

To our knowledge, this is the largest published series of NMM. *N. meningitidis* was the third most frequent cause of neonatal bacterial meningitis in our series in France (2.8%), then *L. monocytogenes* (2.4%). All cases occurred in full term newborns, and nearly all were late-onset meningitis (96%), with only one occurring at day 4. These results are similar to those found by Shepard et al. This specific distribution of *N. meningitidis* as a late-onset infection might be explained by the potential mode of transmission of this bacterium. Most neonatal meningitis agents are feto-maternal or digestive transmitted and are responsible for early- as well as late-onset infections. The predominant late-onset occurrence of NMM might be related to postnatal respiratory transmission (*N. meningitidis* is part of the normal flora of the upper respiratory tract), by direct contact or by droplets.

CSF culture was positive for nearly all of our patients (91%); 2 had positive findings on PCR and capsular antigen detection in CSF. As in other studies, the combination of different techniques (CSF culture, CSF Gram staining, and also PCR and capsular antigen detection in CSF) improved diagnostic accuracy.

As in other studies in neonates or older children in France,

serogroup B accounted for most of our NMM cases (78% overall), with rates of serogroup C (13%) and others (9%) also similar. Although limited typing data are available, they suggest phenotypic and genotypic heterogeneity and 45% of isolates did not belong to invasive ccs.

The epidemiology of bacterial meningitis has changed in the last decades, after the introduction of routine immunization with Haemophilus influenzae type b, pneumococcus and meningococcus C. Furthermore, implementation of food hygiene measures to prevent *L. monocytogenes* infection divided by 10 the incidence of *L. monocytogenes* neonatal infections between 1984 and 2006 in France.

The main limitation of our study is some missing data. However, our study was based on a large prospective cohort and included a large number of well-documented cases. In conclusion, in this large prospective French cohort of neonatal bacterial meningitis, *N. meningitidis* was the third most frequent bacterial species found. This disease occurred only in term neonates, and infections were mainly late onset. Serogroup B was implicated in 78% of cases, and all strains were susceptible to cefotaxime but not 12% showed reduced susceptibility to aminopenicillin or to penicillin G.

**ACKNOWLEDGMENTS**

The authors thank all pediatricians and microbiologists of the “Observatoire National des Meningites” who participated in this study.

**REFERENCES**


**STREPTOCOCCUS GALLOLYTICUS SUBSP. PASTEURIANUS INFECTION IN A NEONATAL INTENSIVE CARE UNIT**

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**Abstract:** We report nosocomial transmission of *Streptococcus galolyticus* subsp. *pasteurianus* among 3 neonates, 1 of whom died. Genome analysis of the strains showed a specific pattern of metabolic and regulatory functions as well as of expressed antigens and antibiotic resistance genes that might have contributed to their specific virulence.

**Key Words:** Streptococcus galolyticus subsp. pasteurianus, neonatal, transmission, genome sequencing, virulence factors

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**Streptococcus gallolyticus** subsp. **pasteurianus** is a Lancefield group D streptococcus, formerly known as **Streptococcus bovis** biotype II. By means of 16S rRNA gene sequencing, the **S. bovis** conglomérator could be differentiated into the genomospecies **S. gallolyticus** subsp. **gallolyticus** (former biotype I), **Streptococcus infantarius** subsp. **coli** (former biotype II/1) and **S. gallolyticus** subsp. **pasteurianus** (former biotype II/2).2 **S. gallolyticus** subsp. **pasteurianus** specifically is associated with meningitis, whereas **S. gallolyticus** subsp. **gallolyticus** is linked to endocarditis and colon cancer.4 We present a cluster of 3 neonatal cases with **S. gallolyticus** subsp. **pasteurianus** and determined the strains’ virulence characteristics with genome sequencing.

**Patient 1**

A preterm male neonate was born at 30 weeks postmenstrual age, after premature rupture of membranes and preterm labor. On day 7, the infant became hemodynamically unstable. Vancomycin and amikacin were initiated for suspected late-onset sepsis. Cer-ebrospinal fluid showed increased white blood cell count and protein concentration but culture remained sterile. Blood culture grew **S. gallolyticus** subsp. **pasteurianus** suscepti ble to penicillin. The patient recovered and was discharged at a postnatal age of 57 days.

**Patient 2**

A second preterm male neonate was born at postmenstrual age of 32 weeks because of maternal HELLP (hemolysis, elevated liver enzymes, low platelet count) syndrome and oligohydramnios.

On day 34, he suddenly deteriorated, developed septic shock, diffuse intravascular coagulation, progressive metabolic acidosis, respiratory failure and pulmonary hemorrhage requiring mechanical ventilation. He died within 2 hours. From 2 blood cultures **S. gallolyticus** subsp. **pasteurianus** was grown, susceptible to penicillin, amoxicillin, gentamicin and vancomycin and resistant to erythromycin.

Because patients 1 and 2 were in close proximity on the Neonatal Intensive Care Unit, nosocomial transmission was sus-pected. Therefore, contact isolation was initiated for patient 1, the patients’ rooms were additionally cleaned and disinfected, extra focus on hand hygiene compliance and rectal screening on the ward were initiated. As a result, patient 3 was detected.

**Patient 3**

A male with intrauterine growth restriction in monochori-onic twins was born at 30-week postmenstrual age. During his stay in the Neonatal Intensive Care Unit, he developed 2 episodes of necrotizing enterocolitis. On day 64, rectal screening culture was positive for **S. gallolyticus** subsp. **pasteurianus**. At that time, the boy was asymptomatic and was nursed in isolation up to discharge.

**METHODS**

The **S. gallolyticus** subsp. **pasteurianus** strains were identi-fied by means of matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry (Bruker Daltonics, Bremen, Germany).

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**TABLE 1. Major Functions Encoded by Genes* Specific to S. pasteurianus Strains ATCC 43144 and the Strain from the Second Patient (HC-2909-2)**

<table>
<thead>
<tr>
<th>Functional Categories</th>
<th>Strain ATCC 43144</th>
<th>HC-2909-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon metabolism</td>
<td>Allulose PTS permease and metabolism</td>
<td>Putative sugar ABC transporter system</td>
</tr>
<tr>
<td></td>
<td>Keto deoxysucrose permease</td>
<td>Oligo-1,6-glucosidase</td>
</tr>
<tr>
<td></td>
<td>Dihydroyxacetone operon</td>
<td>Alpha galactosidase and sucrose-specific PTS system</td>
</tr>
<tr>
<td></td>
<td>Alcohol dehydrogenase</td>
<td>PTS system, ascorbate family</td>
</tr>
<tr>
<td>DNA metabolism and regulators</td>
<td>CRISPR-cas (type 2-B)</td>
<td>l-Fucose and l-fucosyl oligosaccharides utilization operon</td>
</tr>
<tr>
<td></td>
<td>DNA cytosine methyltransferase</td>
<td>CRISPR-cas (type 2-A)</td>
</tr>
<tr>
<td></td>
<td>DNA-binding protein</td>
<td>TCS (2×)</td>
</tr>
<tr>
<td>Surface polysaccharides</td>
<td>Capsular polysaccharide†</td>
<td>CovR CovS, virulence TCS</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>Glycosyl transferase</td>
<td>Transcription regulator coupled with an ABC transporter</td>
</tr>
<tr>
<td></td>
<td>l-Antibiotic, Nisin U biosynthesis</td>
<td>Type 2 restriction modification system</td>
</tr>
<tr>
<td></td>
<td>l-Antibiotic ABC transporter</td>
<td>DNA-cytosine methyltransferase</td>
</tr>
<tr>
<td>Ion transport</td>
<td>trk K⁺ uptake</td>
<td>Capsular polysaccharide†</td>
</tr>
</tbody>
</table>

*Phages, remnant phages and mobile genomic elements are not reported.
†Corresponds to 2 different capsular operons.
‡Present but inactivated by a frameshift mutation in the ATCC 43144 strain.
±2-component regulatory system.
Bold indicates the main genes associated with virulence potency and antimicrobial resistance that are discussed in the text.
RESULTS

All 3 strains belonged to the same PFGE type A, indicating clonality knowing that PFGE is the most discriminative technique for this species. Moreover, clustering of cases with this uncommon invasive pathogen on the same hospital ward in this short 3-week period is a strong indication of nosocomial transmission.

Genome sequencing showed that the 3 strains were virtually identical, confirming PFGE data: the patients’ strains differed by maximum 4 SNPs from each other. The sequence of the 16S rRNA genes was 100% identical over the whole length (1462 bp) to that of the published ATCC strain S. galloolyticus subsp. pasteurianus 43144. Whole-genome comparisons with strain ATCC 43144 confirmed the species identification and showed that the patients’ strains are rather distantly related to the ATCC strain with 0.4% of polymorphism. Interestingly, analysis of the SNP distribution by SyntView (http://genopole.pasteur.fr/SyntView/flash/Streptococcus_pasteurianus/SynWeb.html) along the genome alignment shows an uneven distribution of SNP density with alternate patterns of regions of high and low SNP density, suggesting a high recombination rate involving large genomic regions as previously described in Streptococcus agalactiae.13

Both the ATCC 43144 and the patients’ strains possessed specific genes. However, the 3 patient strains analyzed here showed unique features possibly associated with their fitness and virulence (Table 1).

DISCUSSION

We analyzed the specific genes of the 3 patients’ S. pasteurianus strains in search for their virulence potency. These strains had a complete operon for the utilization of L-fucose and L-fucosyl oligosaccharides. Fucose, a mucin component is both a carbon source and a signal molecule. Blast search revealed that this locus is missing in sequenced S. pasteurianus and S. galloolyticus strains but that it is present in Streptococcus suis pointing at a recent acquisition of the locus by lateral gene transfer. This locus encodes a protein highly similar to the α-L-fucosidase from Bifidobacterium. Likewise, Stahl et al14 showed enhanced survival in the piglet gut of Campylobacter jejuni strains that use L-fucose as a substrate of growth. The CovRS 2-component system regulating the transition from commensalism to invasiveness in group A and group B streptococci is missing in the ATCC 43144 strain but is present in these S. galloolyticus isolates. Furthermore, the ATCC 43144 strain expresses a different capsule operon and a different locus for the synthesis of bacteriocin and competence compared with the sequenced strains. How the specific gene content of these 3 strains might contribute to their virulence remains to be analyzed. Probably more significant is the identification of antibiotic resistance genes in all 3 strains: tet(M): tetracycline resistance determinant, ermB: an adenine N-6-methyltransferase conferring resistance to erythromycin, aadE: a streptomycin aminoglycoside 6-aminohydrolase, and aphA3: a putative spectinomycin adenylyltransferase.

All these genotypic resistance determinants were associated with minimum inhibitory concentrations of at least 64 μg/mL, suggesting a hypothetical resistance to these antibiotics for all 3 strains.

In conclusion, nosocomial transmission of S. galloolyticus subsp. pasteurianus in a Neonatal Intensive Care Unit resulted in screening and isolation measures. No further infections/colonizations with this species arose. This shows the importance of awareness of possible transmission of microorganisms other than the familiar multidrug-resistant organisms.

Genome analysis of the 3 strains showed a specific pattern of metabolic and regulatory functions as well as of expressed antigens that might have contributed to the specific virulence of these strains. In addition, the number of antibiotic resistance genes present in its genome possibly witnesses a long history of interaction with humans in a disease or hospital context, which also might be associated with an increased capacity for dissemination.

REFERENCES

infection was reported in 4 unimmunized causes by
B. pertussis
simultaneous the APV comparator (Lederle/Takeda 4 component) in a study per- 
cased by
infection, one WPV (manufactured by
ses were performed. With regards to prevention of whooping cough
intervals with an unspecified whole-cell pertussis component vac-
majority of cases,
B. parapertussis
B. pertussis
infection was followed by
infections in 4 children and in the remaining great
parapertussis
B. pertussis
infection that was followed by
infections. 7,8 However, as demonstrated in our case, when
confronted with an individual patient, illness caused by
B. parapertussis
infection is clinically indistinguishable from illness caused by
B. pertussis.
Importantly, B. pertussis infection or vaccination with a whole-cell pertussis vaccine does not protect against whooping cough caused by
B. parapertussis
and infection with
B. parapertussis
does not appear to induce protection against disease caused by
B. pertussis.3,5,6,8 Moreover, there is lack of evidence for APV in use today to protect against
B. parapertussis
disease. Therefore, there is a need for application of specific diagnostic tests to discriminate between
B. pertussis and
B. parapertussis
infections as part of pertussis surveillance accompanying immunization programs.

**DISCUSSION**

In comparative cohort studies, signs and symptoms of cough illness caused by
B. parapertussis infection are on average milder and of shorter duration when compared with those caused by
B. pertussis.
However, as demonstrated in our case, when confronted with an individual patient, illness caused by
B. parapertussis
infection is clinically indistinguishable from illness caused by
B. pertussis.
Importantly, B. pertussis infection or vaccination with a whole-cell pertussis vaccine does not protect against whooping cough caused by
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disease. Therefore, there is a need for application of specific diagnostic tests to discriminate between
B. pertussis and
B. parapertussis
infections as part of pertussis surveillance accompanying immunization programs.

Physicians confronted with children with cough illness must consider the possibility of
B. parapertussis
infection irrespective of the patient’s immunization history and/or previously diagnosed whooping cough by
B. pertussis
infection. In the absence of a specific serologic test to confirm
B. parapertussis infection, a reliable diagnosis relies on demonstration of the organism by culture or PCR.3

**REFERENCES**