Scripta Scientifica Medica, vol. 36 (2004), pp 43-44

LISTERIOSIS IN NEONATES - A MICROBIOLOGICAL STUDY

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ABSTRACT

The study shows the important role of *Listeria monocytogenes* as an etiological agent of 3 cases of neonatal infection in Varna City during the period 2002 - 2003. The etiological diagnosis is based on detection the microorganism in blood cultures, nasopharyngeal and other secretions, obtained from the newborns. *L. monocytogenes* is isolated and identified by conventional methods. Some microbiological laboratorial aspects are reported. *Listeria monocytogenes* is one of the six species of genus Listeria. It is a human pathogen of high public health concern. Diseases, caused by this microorganism affect some groups of patients, who are especially susceptible - elderly patients, immunocompromised patients, pregnant women and neonates. *L. monocytogenes* is predominantly transmitted by the consumption of contaminated foods. In pregnant women, *L. monocytogenes* often causes an influenza - like illness that, if untreated may lead to infection of the fetus, resulting in abortion, stillbirth or premature birth, because it is able to cross the placenta (6). Neonatal infection is divided into early (less than 2 days old), intermediate (3-5 days old) and late (more than 5 days old) onset disease. Early neonatal listeriosis is predominantly a septicaemic illness, contracted in utero. In contrast, late neonatal infection is predominantly meningitic and may be associated with hospital cross infection (4). Early onset disease represents a spectrum of mild to severe infection, which can be correlated with the microbiological findings. The main sites of isolation are blood, superficial sites and amniotic fluid, less commonly gastric aspirate, cerebrospinal fluid (CSF) and high vaginal swabs. The main site of isolation of *L. monocytogenes* for the late onset disease is CSF commonly and rarely blood.

Key words: neonatal listeriosis, isolation of L.monocytogenes, antimicrobial susceptibility

The aim of this study was microbiological investigation of cases of neonatal infection in Varna Hospital of Obstetrics and Gynecology and determination the antimicrobial susceptibility, morphologic, cultural and biochemical characteristic of *L. monocytogenes* isolates, which were proved to be the etiological agent.

MATERIAL AND METHODS

Blood, nasopharyngeal, eye, ear and umbilical secretions, lochial secretions, gastric aspirates, skin swabs, meconium, CSF and blood samples for serodiagnosis were examined. The following media were used: 5% sheep blood agar, chocolate agar, glucose broth, MH agar. Strains: *L. monocytogenes*, isolated from 3 newborns - 10 strains.
Isolation and identification of *L. monocytogenes* strains to genus and species level were done by conventional methods (1, 2).
The clinical specimens were directly plated on 5% sheep blood agar, chocolate agar, Levin agar and glucose broth and cultured at 35° for 24 h. Samples for blood culture were inoculated into conventional blood culture broth. Suspected colonies - small, 1-2mm, smooth and gray, were transferred to 5% sheep blood agar and incubated for 18 hours at 35° for further work up. The CAMP test was used for verifying the hemolytic activity. It was performed with beta hemolytic *S. aureus* strain (ATCC 25923). Biochemical differentiation was done by conventional tests: tubes with mannitol, rhamnose and xylose were inoculated and cultured at 37° for 18 hours. Antimicrobial susceptibility testing was performed by disk-diffusion method, according to the NCCLS recommendations. Widal agglutination was used for serodiagnosis.

RESULTS AND DISCUSSION

Three newborns from the neonatal unit in Varna Hospital of Obstetrics and Gynecology with clinical symptoms of septicemia were tested. Two of the preterm babies were born of mothers, who were febrile with influenza - like illness 2-3 days before delivery. During the process of testing of these 3 cases, bacteriological analysis of various clinical specimens, obtained from the neonates and mothers was performed. From the babies were taken several times the above mentioned clinical specimens. Ten isolates of *L.*

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monocytogenes I serogroup, serotype 1/2a were identified from the three newborns. The highest percentage of isolation was from blood cultures (from the three newborns) and nasopharyngeal secretions (from the two of them). *L. monocytogenes* was isolated also from skin, eye and ear secretions (table 1). The lochial cultures of the mothers were negative.

There was no significant difference in the pattern of antimicrobial susceptibility and resistance of *L. monocytogenes*.

The serological test "Widal agglutination" was used to detect and measure antibody levels in sera, obtained from the women. In only one woman was found agglutination titre 1:360, without increasing after two weeks. No significant

Table 1. Isolation of L. monocytogenes from various clinical specimens

Case No	nasopharyngeal secretion	eye secretion	ear secretion	skin swabs	gastric aspirate	meconium	blood	CSF
I	+	+	-	-	-	-	+	-
II	+	-	-	+	-	-	+	-
III	-	-	+	-	-	-	+	-

We used several tests for identification: Gram stain morphology - the isolates were Gram positive rods; positive catalase reaction; the observation of characteristic motility - umbrella shaped pattern that developed after over night incubation of the cultures into a tube of semisolid agar at room temperature; ability to grow at 4° C. We observed a hemolytic activity of the isolates L.monocytogenes on 5% blood sheep agar. The hemolytic zones were narrow and under the removed colonies. The hemolysis by the isolates was enhanced near the growth of S. aureus.

All isolates were mannitol and xylose negative and rhamnose positive. Serotype determination showed that all strains *L. monocytogenes* are serogroup I, serotype 1/2a.

In vitro the isolates were found susceptible to Penicillin, Ampicillin, Piperacillin, Ampicillin/sulbactam, Gentamicin, Cotrimoxazole - 80-100%. The resistance to Cephalosporins (incl. III generation) was 100% (table 2). We compared these results to antimicrobial susceptibility of 50 *L. monocytogenes* strains from various clinical specimens collected during 1990-1998.

Table 2. Susceptibility and resistance rates of L. monocytogenes isolates

Antimicrobial agent	S (%)	R(%)
Penicillin	80	20
Ampicillin	100	0
Ampicillin / Sulbactam	100	0
Piperacillin	100	0
Cefoperazone	0	100
Cotrimoxazole	100	0

serologic reaction was found in the other women.

The report proves the important etiological role of *L. monocytogenes* in cases of neonatal septicemia. The isolation of small Gram-positive, catalase-positive rods with a narrow zone of beta hemolysis especially from blood and CSF must be used as a strong presumptive evidence of listeriosis (5). The isolates show high rates of susceptibility to most commonly used antimicrobials - Penicillin, Ampicillin, Gentamicin. Even more, some authors mention that antimicrobial susceptibility and resistance of *L. monocytogenes* stay relatively stable for many years (7). Cephalosporins are not effective in vitro, because of the natural resistance of *L. monocytogenes* to this group of antimicrobials. They should never be administrated when listeriosis is suspected (3, 7).

Although the serological tests are not recommended at the present time, because of antigenic cross-reactivity between *L. monocytogenes* and other gram-positive bacteria, these tests can be used as additional tests in diagnostic process and must be taken into consideration only after comparing to the clinical symptoms and monitored in time.

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