mid mediated beta-lactamase BIL-1. *J Antimicrob Chemother* 1992; 30: 119–27.
8. Gonzalez Leiza M, Perez-Diaz JC, Ayala J et al. Gene sequence and biochemical characterization of FOX-1 from *Klebsiella pneumoniae*, a new AmpC-type plasmid-mediated beta-lactamase with two molecular variants. *Antimicrob Agents Chemother* 1994; 38: 2150–7.
9. Jarlier V, Nicolas MH, Fournier G, Philippon A. Extended broad-spectrum beta-lactamases conferring transferable resistance to newer beta-lactam agents in Enterobacteriaceae: hospital prevalence and susceptibility patterns. *Rev Infect Dis* 1988; 10: 867–78.
10. Cormican MG, Marshall SA, Jones RN. Detection of extended-spectrum beta-lactamase (ESBL)-producing strains by the Etest ESBL screen. *J Clin Microbiol* 1996; 34: 1880–4.
11. Jacoby GA, Sutton L. Properties of plasmids responsible for production of extended-spectrum beta-lactamases. *Antimicrob Agents Chemother* 1991; 35: 164–9.
12. Vatopoulos AC, Philippon A, Tzouvelekis LS, Komninou Z, Legakis NJ. Prevalence of a transferable SHV-5 type beta-lactamase in clinical isolates of *Klebsiella pneumoniae* and *Escherichia coli* in Greece. *J Antimicrob Chemother* 1990; 26: 635–48.
13. Philippon A, Labia R, Jacoby G. Extended-spectrum beta-lactamases. *Antimicrob Agents Chemother* 1989; 33: 1131–6.
14. Rice LB, Bonomo RA. Genetic and biochemical mechanisms of bacterial resistance to antimicrobial agents. In: Lorian V, ed. *Antibiotics in Laboratory Medicine*. Baltimore, MD: Williams & Wilkins, 1996: 453–501.
15. Martinez-Martinez L, Pascual A, Jacoby GA. Quinolone resistance from a transferable plasmid. *Lancet* 1998; 351: 797–9.
16. Pfaller MA, Hollis RJ, Sader HS. Chromosomal restriction fragment analysis by pulsed-field gel electrophoresis. In: Isenberg HD, ed. *Clinical Microbiology Procedures Handbook*. Volume (Suppl 1). Washington, DC: American Society for Microbiology, 1994: 10.5c.1–12.
17. Tenover FC, Arbeit RD, Goering RV, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed- field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* 1995; 33: 2233–9.
18. Meyer KS, Urban C, Eagan JA, Berger BJ, Rahal JJ. Nosocomial outbreak of *Klebsiella* infection resistant to late- generation cephalosporins [see comments]. *Ann Intern Med* 1993; 119: 353–8.
19. Bush K, Jacoby GA, Medeiros AA. A functional classification scheme for beta-lactamases and its correlation with molecular structure. *Antimicrob Agents Chemother* 1995; 39: 1211–33.
20. Bradford PA, Cherubin CE, Idemyor V, Rasmussen BA, Bush K. Multiply resistant *Klebsiella pneumoniae* strains from two Chicago hospitals: identification of the extended-spectrum TEM-12 and TEM-10 ceftazidime-hydrolyzing beta-lactamases in a single isolate. *Antimicrob Agents Chemother* 1994; 38: 761–6.
21. Jacoby GA, Carreras I. Activities of beta-lactam antibiotics against *Escherichia coli* strains producing extended-spectrum beta-lactamases. *Antimicrob Agents Chemother* 1990; 34: 858–62.
22. Soilleux MJ, Morand AM, Arlet GJ, Scavizzi MR, Labia R. Survey of *Klebsiella pneumoniae* producing extended-spectrum beta-lactamases: prevalence of TEM-3 and first identification of TEM-26 in France. *Antimicrob Agents Chemother* 1996; 40: 1027–9.
23. Jacoby GA. Genetics of extended-spectrum beta-lactamases. *Eur J Clin Microbiol Infect Dis* 1994; 13: S2–11.
24. Jacoby GA, Bush K. *Amino acid sequences for TEM, SHV and OXA extended-spectrum beta-lactamases.* http://www.lahey.org/studies/webt.htm., 1998.
25. Nuesch-Inderbinen MT, Hachler H, Kayser FH. New system based on site-directed mutagenesis for highly accurate comparison of resistance levels conferred by SHV beta-lactamases. *Antimicrob Agents Chemother* 1995; 39: 1726–30.
26. Livermore DM. Beta-lactamases in laboratory and clinical resistance. *Clin Microbiol Rev* 1995; 8: 557–84.
27. Rice RJ, Knapp JS. Antimicrobial susceptibilities of *Neisseria gonorrhoeae* strains representing five distinct resistance phenotypes. *Antimicrob Agents Chemother* 1994; 38: 155–8.
28. Rice LB, Willey SH, Papanicolaou GA et al. Outbreak of ceftazidime resistance caused by extended-spectrum beta- lactamases at a Massachusetts chronic-care facility. *Antimicrob Agents Chemother* 1990; 34: 2193–9.
29. Schiappa DA, Hayden MK, Matushek MG et al. Ceftazidime-resistant *Klebsiella pneumoniae* and *Escherichia coli* bloodstream infection: a case-control and molecular epidemiologic investigation. *J Inf Dis* 1996; 174: 529–36.

Neonatal listeriosis in Algeria: the first two cases

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Listeriosis is regularly reported in Europe and North America [1] but it is rare in Africa and other developing countries. In North Africa, only seven cases have previously been reported in Algeria and six cases in Tunisia [3].

Listeriosis is a food-borne disease which occurs at epidemic or sporadic infections. Neonatal listeriosis is divided into early onset with manifestations of septicemia whereas meningitis is most often acquired at late onset [1]. The early-onset form is

apparent within the first 2 days of life and results from intrauterine infection, whereas the late-onset form occurs from several days to weeks after birth and may result from acquisition of organisms during passage through the birth canal or nosocomial transmission [1].

We report the first two cases of neonatal listeriosis in Algeria which occurred at the same maternity hospital within an interval of 29 days. Microbiologic and random polymerase chain reaction (PCR) results suggested a common source of infection.

A term male infant weighing 3300 g was delivered to a healthy 34 years-old gravida 4, para 2 mother by spontaneous vaginal delivery after an uncomplicated 8-h labour. She was admitted 6 h before delivery. Membranes were artificially ruptured 2 h before delivery revealing abnormal amniotic fluid. The pregnancy had been uncomplicated except for an influenza-like illness and green vaginal discharge a week before delivery. The mother lived on a farm 50 km from Algiers and the family consumed milk from their own cow daily.

At 24 h of age, the infant was admitted to the neonate intensive care unit suffering from respiratory distress syndrome. He presented with septicemia, conjunctivitis and a localized cutaneous rash on the lower limbs. After lumbar puncture, which was clear, blood culture and uroculture, the infant was empirically treated with intravenous ampicillin, 100 mg/kg per day divided into four doses and intramuscular gentamicin, 5 mg/kg per day divided into two doses.

Listeria monocytogenes was isolated from blood (S1) and urine (S2). Following this result, cervical and vaginal swabs were obtained from the mother 5 days after the delivery and revealed *L. monocytogenes* (S3) associated with *Streptococcus agalactiae*.

The treatment of the infant was continued and he was discharged after 21 days. He was found to be normal at the 1-year follow-up examination.

In the same delivery room, 29 days after case 1, a term male infant weighing 2850 g was born, to a healthy 22-year-old primigravida mother by spontaneous vaginal delivery after an uncomplicated 14-h labour. She was admitted 20 h before delivery. Membranes spontaneously ruptured 6 h before delivery revealing abnormal amniotic fluid. The pregnancy had been uncomplicated except that the mother, who is a citizen and lives in Algiers, had fever 2 days before delivery.

The infant was admitted to the neonate intensive care unit 42 h after birth, suffering from convulsions. He presented signs of meningitis with a generalized cutaneous rash and a temperature of 35 °C, measured rectally.

Blood culture and a lumbar puncture were carried out. The cerebrospinal fluid revealed the following values: leucocyte count, 300/mm^3, with 80% polymorphonuclear cells and 20% lymphocytes; glucose level 20 mg/dL (blood glucose level 64 mg/dL); protein concentration 16.2 mg/dL; and the Gram-stained smear showed Gram-positive bacilli.

Despite the antibiotic treatment, intravenous ampicillin 200 mg/kg per day divided into four doses and intramuscular gentamicin 7.5 mg/kg per day divided into two doses, the infant died 13 h later.

Listeria monocytogenes was isolated from the cerebrospinal fluid (CSF) (S4) and blood (S5) of the baby, and from the vaginal secretions (S6) of the mother, 7 days after delivery. *Listeria monocytogenes* was identified on the basis of Gram's stain, colonial morphology, motility, catalase reaction, a positive Voges Prauskauer test, bile esculin hydrolysis and fermentation of glucose, lactose, sucrose, maltose and rhamnose. This identification was confirmed at the Service de bactériologie médicale, Institut Pasteur, Algerie (Dr K. Rahal).

The isolates were sent to the Centre National de Référence (CNR) for listeriosis, Institut Pasteur, Paris (Dr J. Rocourt), for serotyping and phage-typing.

We studied the six isolates of *L. monocytogenes* and one unrelated strain of *L. monocytogenes* serotype 1/2a by means of random PCR analysis at Laboratoire de microbiologie, Hopital Robert Debré, Paris (Dr E. Bingen). Two PCR primers 5′GTTTCGCTCC3′ (primer A) and 5′TGGGAGGTGTATAGTCTA3′ (primer B) were used for each random PCR analysis as described previously [4]. Isolates that differed by more than one fragment were considered sufficiently divergent to warrant a separate strain designation.

The six isolates of *L. monocytogenes* were shown to be serotype 1/2 a and unstable on phage typing. The random PCR analysis using the two primers A and B revealed that the six clinical isolates of *L. monocytogenes* shared the same pattern, which was different from the unrelated strain (Figure 1).

The epidemiology of neonatal infections in developing countries is not well established. Several fragmentary studies show that Gram negative bacilli (*E. coli*) are the most frequent agents, and this is true in Algeria (unpublished data). The first case of listeriosis was reported in 1968 [2] when *L. monocytogenes* was isolated from CSF of a 4-year-old immunocompromised child. Two other cases of meningitis with *L. monocytogenes* serotype 4 were reported in 1969. Between 1985 and 1987 four other cases were reported (R. Bellouni, thesis 1991). Two strains of *L. monocytogenes* serotype 1/2b and 4b were isolated from the placenta of two mothers but no samples were collected from their infants. The two last strains of *L. monocytogenes* serotype 4b have been isolated from blood culture and CSF in two immunocompromised adults. In Tunisia four cases of neonatal listeriosis were reported, for which the suspected source was milk [3].

In our study the results of random PCR suggest a common source of contamination which may result from a food-borne illness or a nosocomial infection. In the first case, raw milk was probably the source of contamination but no obvious source could be found for the second mother. She was a citizen living in another town. In fact it was difficult to establish the source

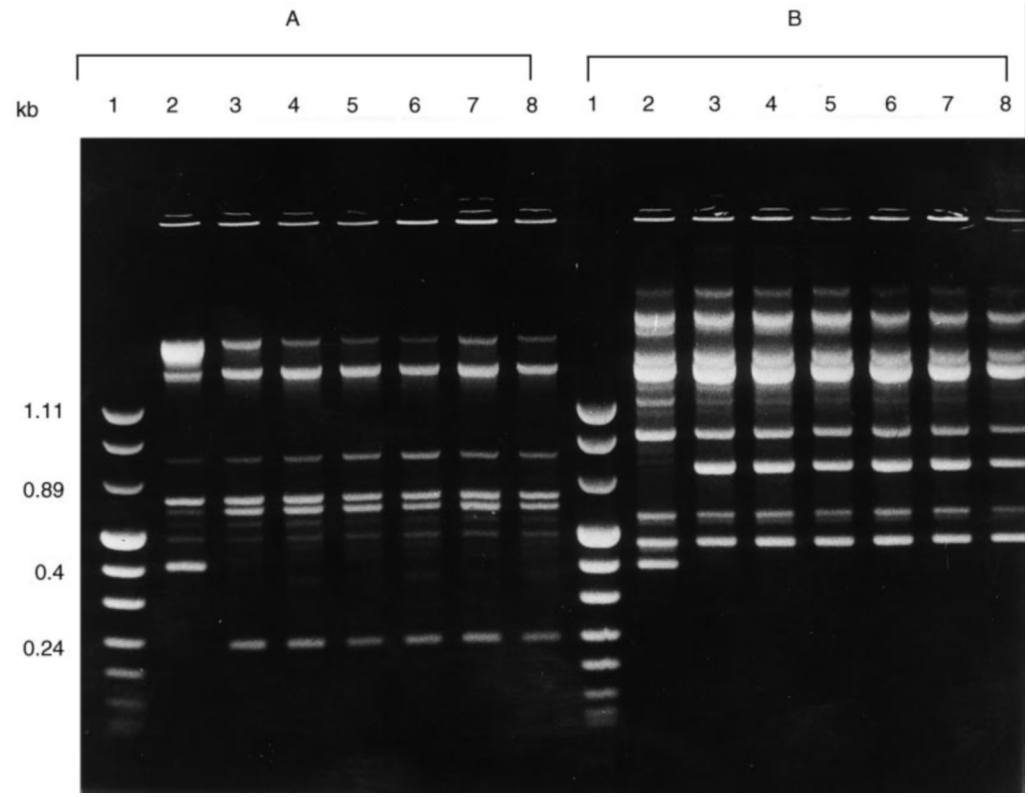

Figure 1 DNA fingerprinting of *L. monocytogenes* by random polymerase chain reaction analysis with the two primers A and B. Lane 1, size marker; Lane 2, unrelated strain; Lanes 3 to 5, isolates from case 1 infant: blood culture (S1), urine sample (S2), vaginal sample (S3), respectively. Lanes 6 to 8, isolates from case 2 infant: cerebrospinal fluid (CSF) (S4); blood culture (S5); vaginal sample (S6), respectively. Numbers on left are reference molecular size markers.

because of the lag time between the consumption of the contaminated food and the subsequent diagnosis of the infection.

Nosocomial transmission from the first mother to the second by some inadequately decontaminated material could be suspected. Both mothers came to the maternity hospital for follow-up visits during the same periods. Listeria is a ubiquitous bacterium which can survive for several weeks in the environment [1]. However, the absence of other cases at the same maternity hospital and the 29-day interval between the two cases suggest that both mothers may have had a food-borne illness with a common strain.

Nosocomial cross-acquisitions of listeria have been observed during periods of a few hours or days [5,6]. The transmission may occur in delivery rooms or neonate intensive care units, especially through resuscitation equipment.

The short time period in the two cases described in this report, suggests that both infections occurred *in utero* through blood transmission. *Listeria monocytogenes* is not a habitual genital host, but can be isolated from lochia for a few days before and after delivery. In the absence of epidemiological investigations the source of contamination remains hypothetical.

Serotyping of *L. monocytogenes* is not very discriminant as almost all strains belong to a small number of serotypes [1]. Phage-typing is more discriminating and could be applied to a large numbers of isolates, but many isolates remain untypeable. The reason for the instability of phage-typing could not be explained for our strains but may result from their storage. Molecular typing methods are highly discriminant. Random PCR analysis was found to be comparable to conventional phage-typing [7] but quicker and simpler for epidemic investigations, whereas Pulsed-field Gel Electrophoresis (PFGE) was found to be more time-consuming but highly discriminant when used in association with arbitrarily primed (AP)-PCR [8].

During the investigations of the French listeriosis outbreak in 1992, phage-typing and PFGE were found to be the most discriminating methods [9]. Random PCR had previously been found to be simple, rapid and useful for various microbial isolates [10].

As the food industry is not very developed in North Africa, listeriosis must be evaluated by studies of carriage in Algeria and particularly in rural areas. Despite the fact that listeriosis is still rare in our country and may be unrecognised and not diagnosed,

an epidemiologic survey is necessary to evaluate and prevent this illness from affecting persons at risk.

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REFERENCES

1. Schuchat A, Swaminathan B, Broome CV. Epidemiology of human listeriosis. *Clin Microbiol Rev* 1991; 4: 169–83.
2. Benallegue A, Benhassine M, Grangaud JP, Mazouni M. Méningite à *Listeria monocytogenes*. *Algérie médicale* 1968; V: 29–32.
3. Boukadida J, Sboui H, Monastiri K et al. La listériose humaine en Tunisie: deux nouveaux cas chez le nouveau né. *Med Mal Infect* 1994; 24: 117–8.
4. Bingen E, Boissinot C, Desjardins P et al. Arbitrary primed polymerase chain reaction provides rapid differentiation of *Proteus mirabilis* isolates from a pediatric hospital. *J Clin Microbiol* 1993; 31: 1055–9.
5. Campbell AN, Sili PR, Wardle JK. Listeria meningitis acquired by cross infection in a delivery suite. *Lancet* 1981; 3: 752–3.
6. Nelson KE, Warren D, Tomasi AM et al. Transmission of neonatal listeriosis in a delivery room. *Am J Dis Child* 1985; 139: 903–5.
7. Mazurier SI, Audurier A, Marquet-Van Der Meen Notermans S, Wernars K. A comparative study of randomly amplified polymorphic DNA analysis and conventional phage typing for epidemiological studies of *Listeria monocytogenes* isolates. *Res Microbiol* 1992; 143: 507–12.
8. Louie M, Jayaratne P, Luchsinger.I, Devenish J et al. Comparison of ribotyping, arbitrary primed PCR, and pulsed field gel electrophoresis for molecular typing of *Listeria monocytogenes*. *J Clin Microbiol* 1996; 34: 15–9.
9. Jaquet C, Catimel B, Brosch R et al. Investigations related to the epidemic strain involved in the French listeriosis outbreak in 1992. *Appl Environ Microbiol* 1995; 61: 2242–6.
10. Williams JGK, Kubelik AR, Livak J et al. DNA polymorphysms amplified by arbitrary primers are useful as genetic markers. *Nucl Acids Res* 1990; 18: 6531–5.

Therapeutic effects of rifampin and erythromycin in experimental murine brucellosis

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Brucellosis is a serious health problem because of its severe complications and its tendency to be a chronic disease [1]. The most effective and least toxic therapy for human brucellosis is still questionable. Streptomycin, gentamicin, tetracycline, doxycycline, rifampin and trimethoprim–sulfamethoxazole are among the widely used antibiotics in the treatment of human brucellosis. The use of a single antibiotic is not recommended because of reduced efficacy. The combination most frequently recommended is rifampin plus doxycycline for 45 days. Other recommended regimens are streptomycin plus doxycycline and rifampin plus trimethoprim–sulfamethoxazole [2–4]. In spite of these suggested treatments, the relapse rate is still about 10% [1,3].

The treatment of brucellosis in children (under 6–8 years of age) is a problem because of tetracycline accumulation in bones and tooth structures [5]. Brucellosis is also a problem in pregnant women, since for them the usual drugs are contraindicated [2]. Erythromycin is not teratogenic and is safe to be used during pregnancy and for children [5,6]. Therefore, in the treatment of brucellosis, new antibiotics with better activity and a higher degree of safety in pregnant women and children are needed. This study was undertaken to investigate the possibility of erythromycin treatment for brucellosis.

The murine brucellosis model, which is a well-known and commonly used animal model with reproducibility described previously, was used in this study [7–9]. In total, 104 male Balb/c mice (25–30 g) were used. Mice were fed ad libitum with standard mouse diet. In order to estimate the average water consumption per day per mouse, water consumption was measured for a week and daily consumption was calculated for each mouse.

Brucella melitensis M16 was obtained from The Department of Microbiology, School of Veterinary Medicine, Firat University. Minimum inhibitory concentrations (MICs) of antibiotics were