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ARTICLE

A *Chryseobacterium meningosepticum* colonization outbreak in a neonatal intensive care unit

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Abstract
Purpose To report the epidemiologic, bacteriologic, and clinical features of a *Chryseobacterium meningosepticum* outbreak in a neonatal intensive care unit (NICU) of a referral teaching hospital.
Patients and methods From April to October 2002, a strain of *C. meningosepticum* was isolated from four neonates in the NICU. All neonates were colonized in the endotracheal tubes and respiratory secretions, but none of them progressed to clinical infection. Multiple samples were obtained for cultures.
Results Pulsed-field gel electrophoresis (PFGE) of isolates showed them to be representatives of a single strain. Environmental surveillance did not reveal the *C. meningosepticum* source. None of the neonates received specific treatment. The outbreak was only controlled by reinforcement of the usual measures and no additional colonization/infection was confirmed for more than a year after the last case.
Conclusion This study suggests that *C. meningosepticum* colonization in neonates does not necessarily lead to infection and that such colonization outbreaks may be controlled with emphasis on the standard precautions.

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Introduction

Chryseobacterium (formerly *Flavobacterium*) species are aerobic, nonfermenting, oxidase-positive, gram-negative rods, producing a distinct yellow to orange pigment. Ubiquitous in nature, they are found in plants, soil, and water and may survive in chlorine-treated water supplies, hospital environments, and even in the condensation water of space station Mir [1–4]. Humans are colonized via contaminated wet devices, such as wash basins, respirators, intubation tubes, mist tents, humidifiers, incubators for newborns, and ice chests [2, 3]. *Chryseobacterium* species generally possess low virulence and are only very rarely pathogens in humans, infecting newborns and immunocompromised hosts [1, 5, 6]. *C. meningosepticum* appears to be the most pathogenic member of the genus [2], although *C. indologenes* infection has been described [6]. Infections are, as a rule, nosocomial; however, community-acquired bloodstream infections have been reported as well [1, 5, 7].

Chryseobacterium infection may present with a variety of clinical manifestations, including sepsis, meningitis, endocarditis, pneumonia, bacteremia, cellulitis, wound infection, abdominal abscess, sinusitis, bronchitis, epididymitis, dialysis-associated peritonitis, post-traumatic endophthalmitis, and prosthesis-associated septic arthritis [1, 2, 5, 8–11]. Bacteremia, meningitis, and pneumonia seem to be the most common manifestations in neonates [8]. Infections usually affect premature infants [1–3] and often occur as outbreaks. This study describes the epidemiologic, bacteriologic, and clinical features of a *C. meningosepticum* outbreak in a neonatal intensive care unit (NICU).

Patients and methods

Setting

The University General Hospital of Heraklion, Greece, has a NICU of six ventilator beds and a 19-bed, tertiary special care baby unit. Approximately 500 admissions occur annually. During the six-month period from April to October 2002, four neonates in the NICU developed positive cultures from endotracheal tubes and tracheal aspirates.

Environmental screening

Environmental cultures were used to search for the source of the outbreak. Multiple samples were obtained, with particular emphasis on humid places, mechanical ventilators, infant incubators, endotracheal tubes, feeding bottles, sinks, faucets, and door handles. Samples were obtained from the throats and fingers of healthcare workers as well. Samples were inoculated onto Columbia agar with 5% sheep blood, chocolate and MacConkey agar plates, and incubated at 35°C for 48 h.

Microbiology

The samples were processed using standard laboratory protocols. Isolates were identified by the automated system Vitek 2 (bioMérieux, Marcy l'Etoile, France) and the API 20 NE system (bioMérieux). Antimicrobial susceptibility testing was performed by the disk diffusion method following the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS) [12] and the minimum inhibitory concentrations (MICs) of the antibiotics were determined by the E-test method (AB Biodisk, Solna, Sweden). DNA was purified from all six isolates and subjected to polymerase chain reaction (PCR) with the following primers from the 16SrRNA sequence: forward, CAGGCCTAACACATGCAAGTC and reverse, GACGGGCGGTGTGTACAA. The PCR products were sequenced and the derived sequences were compared with the GenBank database, revealing 98% identity with *C. meningosepticum*. Following published definitions of colonization and infection [13], colonization was defined as the presence of *C. meningosepticum* in endotracheal tubes and tracheal aspirates, and infection as the microbiologically proven clinical diagnosis of inflammation.

Molecular typing

Isolates were grown on brain heart infusion agar plates. Cells were removed from the surface of the agar and were resuspended in 2 ml PBS. The cell concentration was adjusted to an absorbance of 0.6–0.9 at 610 nm. A 400-µl aliquot was transferred to a 1.5-ml microcentrifuge tube containing 25 µl of proteinase K (20 mg/ml, Sigma-Aldrich) and mixed gently with 400 µl of 1% SeaKem Gold agarose (FMC). The suspension was dispensed into the wells of plug molds and allowed to solidify at room temperature for 15 min. Cell lysis was achieved in lysis buffer (50 mM Tris, 50 mM EDTA [pH 8.0], 1% sarcosine, 0.1 mg of proteinase K/ml) for 15 min at 54°C, followed by four 20-min washes at 54°C. Restriction proceeded for 20 h, with 40 U of each of the following enzymes separately: RsrII and XhoI at 37°C, BssHII at 50°C. The electrophoresis conditions were set as follows: running buffer TBE 0.5×, initial switch time 2.2 s, final switch time 20 s 6 volts/cm 120° angle, run time 19 h at 14°C [2].

Results

Patients

The patient characteristics are shown in Table 1. Blood and cerebrospinal fluid cultures remained invariably sterile. It is obvious that patients 2, 3, and 4 acquired *C. meningosepticum* without cross-infection with patient 1, as 16 weeks had elapsed between the admission of the former and the discharge of the latter (Fig. 1). None of the affected infants developed clinical manifestations compatible to *C. meningosepticum* infection, none received specific treatment, and all had good outcome and were discharged back to their homes. A prolonged follow-up did not reveal severe infectious episodes in any of these neonates.

Microbiology and molecular biology

All six isolates were identified as *C. meningosepticum* (% id = 99.9%), yielded the same biochemical characteristics with the biocode 2476304 in the API 20 NE system, and had almost identical susceptibility patterns (Table 2). The 16SrRNA sequences confirmed the biochemical identification, showing 98% identity with the corresponding *C. meningosepticum* sequences in the GenBank database. All of the six isolates were compared by pulsed-field gel electrophoresis (PFGE) of chromosomal DNA digests with three different restriction enzymes. The macrorestriction patterns were found to be 100% identical with all three enzymes, except isolate 1, which showed one band difference in the BssHII pattern, clearly indicating that all of the isolates are isogenic of a single strain. The electrophoresis patterns of the strains with the restriction enzymes BssHII and XhoI are depicted in Fig. 2.

Table 1 Characteristics of the neonates colonized with *Chryseobacterium meningosepticum*

Patient, gender	Gestation (weeks)	Birth weight (g)	Age at isolation	Site colonized	Diagnosis
1, female	38	3,100	4 days	Endotracheal tube	Duodenal atresia
2, male	26	1,030	59 days	Tracheal aspirate	Intraventricular hemorrhage, hydrocephalus, shunt, seizures
			67 days	Tracheal aspirate	
			78 days	Endotracheal tube	
3, female	27	800	48 days	Endotracheal tube	Respiratory distress syndrome, *Enterococcus faecalis* sepsis
4, male	39	4,000	3 days	Endotracheal tube	Perinatal stress

Environmental screening

There were 97 other premature and 184 other full-term neonates in the NICU at the time of the outbreak. In none of the cultures obtained from these infants or from surveillance samples or from samples from healthcare workers was *C. meningosepticum* isolated.

Infection control

Standard precaution measures were revised and reinforced, including the use of chlorhexidine gluconate 4% for hand rubbing and of dodecyl dimethyl benzyl-ammonium chloride, tetradecyl dimethyl benzyl ammonium chloride 0.34%, and cetrimide 0.1% for the cleaning of objects. The unit was not thoroughly disinfected and further neonatal admissions were not restricted. A follow-up period of more than 1 year was free from any further cases.

Discussion

C. meningosepticum was first recognized in 1958 as a cause of neonatal meningitis, and outbreaks have occasionally been described since 1961 [14]. Outbreaks usually extend over a period of a few weeks [15, 16], although they may last longer [14, 17], and this was the case in the present study. Outbreaks of *C. meningosepticum* are usually due to transient carriage of the organisms on the hands of healthcare workers, and the original source may be an inanimate reservoir, such as hospital sinks [5, 17]. The exact source often remains obscure in epidemics in NICUs [14, 16], although *C. meningosepticum* has been isolated from faucets, sinks, respiratory therapy equipment, feeding bottles, venous catheter lines, nutritional solutions, contaminated syringes in an ice chest, vials, feeding tubes, flush solutions in arterial catheters, pressure transducers, and antiseptic solutions [1, 7, 15, 17]. We collected numerous samples for environmental surveillance, but failed to isolate

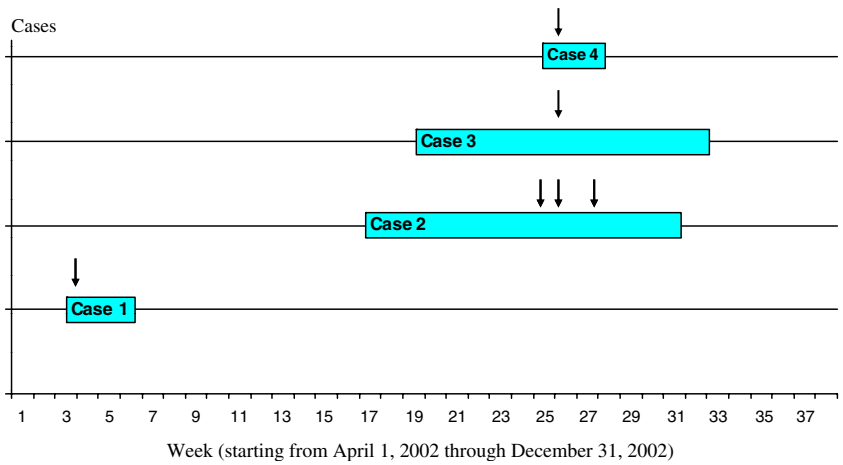

Fig. 1 Chart plotting patient stay in the neonatal intensive care unit (NICU) and the time of confirmed positive cultures (the *arrows* indicate positive cultures)

Table 2 Results of the antibiotic susceptibility testing. The minimum inhibitory concentrations (MICs, μg/ml) are shown in parentheses

Antibiotic	Case 1	Case 2a	Case 2b	Case 2c	Case 3	Case 4
Piperacillin	S (6)	S (6)	S (6)	S (6)	S (6)	S (6)
Piperacillin/ tazobactam	S (4)	S (4)	S (4)	S (4)	S (4)	S (4)
Ticarcillin/clavulanate	R (>256)	R (>256)	R (>256)	R (>256)	R (>256)	R (>256)
Cefotaxime	R (>32)	R (>32)	R (>32)	R (>32)	R (>32)	R (>32)
Ceftriaxone	R (>32)	R (>32)	R (>32)	R (>32)	R (>32)	R (>32)
Ceftazidime	R (>256)	R (>256)	R (>256)	R (>256)	R (>256)	R (>256)
Imipenem	R (>32)	R (>32)	R (>32)	R (>32)	R (>32)	R (>32)
Amikacin	I (24)	I (24)	I (24)	I (24)	I (24)	I (24)
Ciprofloxacin	R (8)	S (0.5)	S (0.5)	S (0.5)	S (0.5)	S (0.5)
Co-trimoxazole	S (0.38)	S (0.38)	S (0.38)	S (0.38)	S (0.38)	S (0.38)

I = intermediate; R = resistant; S = sensitive

C. meningosepticum in any of these. Person-to-person spread is unusual, as manifested by the low rates of infection among neonates housed in adjacent bassinets [8].

In this outbreak, antibiograms and the MICs of the six isolates were almost identical. The results from disk diffusion methods may well be unreliable, so broth reference quality microdilution tests should be performed [1, 2, 8]. The strain identity was genotypically confirmed by PFGE, a method already applied for the molecular typing of multiple isolates [2]. Using three restriction patterns, five of our isolates exhibited 100% identity, demonstrating their common clonal origin and only isolate 1 diverged by a single band in the BssHII restriction pattern. Although the same isolate demonstrated a low-level resistance to ciprofloxacin, the overall similarities suggests the close genetic relatedness of all of the isolates of this study. In our study, only piperacillin and co-trimoxazole were effective against all strains. Clinical isolates of *C. meningosepticum* produce extended spectrum beta-lactamases, and are resistant to penicillins, cephalosporins, and monobactams. In addition, they are long known to be highly resistant to aminoglycosides, tetracyclines, chloramphenicol, erythromycin, clindamycin, and teicoplanin [1, 2, 5, 7, 8, 14, 17–19]. The most

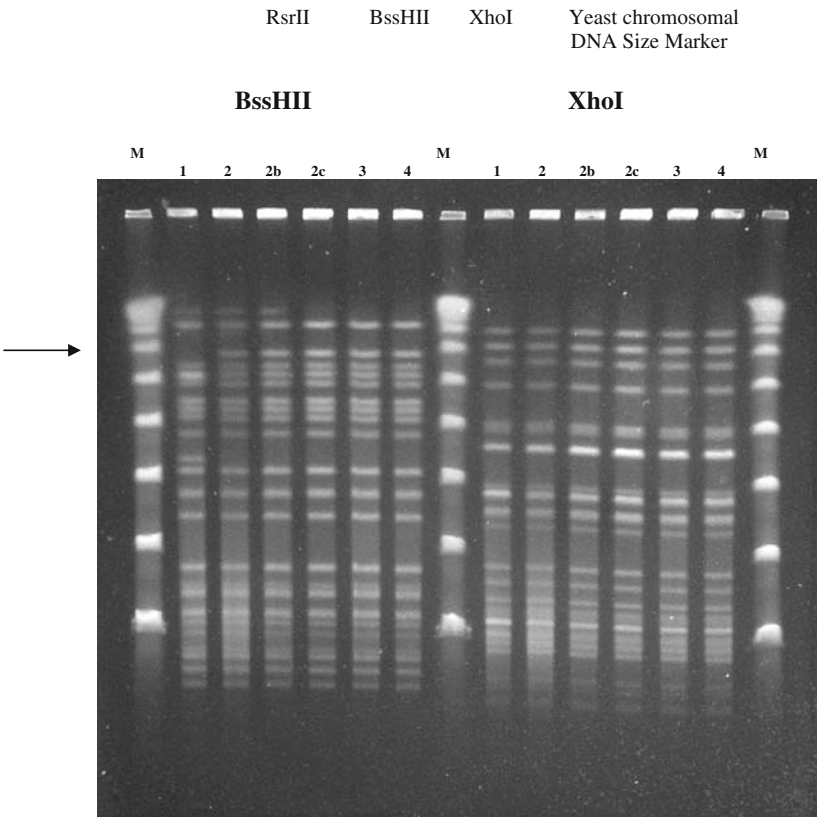

Fig. 2 The pulsed-field gel electrophoresis (PFGE) patterns obtained by BssHII (*left*) and XhoI (*right*). The *arrow* points to the difference of the BssHII pattern of strain 1

active antimicrobials are the newer quinolones and rifampin [2]. Appropriate antibiotic regimens have included combinations of co-trimoxazole, vancomycin, fluoroquinolone, or minocycline with rifampin [1].

Prematurity is a primary risk factor for *C. meningosepticum* infection and half of the infections have involved neonates weighing less than 2,500 g [1]. In this outbreak, two out of four cases weighed less than 1,500 g. The case–fatality rate has been high in neonates, and complications and sequelae are common among survivors [1, 14–16, 20]. By contrast, survival without appropriate antibiotic treatment has been reported in adults [7] and the ratio of colonization versus infection differs widely among studies [14–18]. None of our infants progressed to infection and short- and long-term outcome was excellent in all cases, probably because they were either mature or, in the case of prematurity, they were colonized beyond the first 2 weeks of life. Colonization of the pharynx in clinically healthy babies has been reported in a nursery with an outbreak of *C. meningosepticum* meningitis [3].

Measures that have been used to eradicate *C. meningosepticum* outbreaks in neonatal wards include changing the prescribing policy for empiric antibiotics, restriction of further admissions, and thorough disinfection of the unit [1, 14, 17]. Other studies, however, have shown successful control with milder measures, including alcoholic hand rub after the washing of hands, toileting of babies with sterile instead of tap water, and repair and chlorination of the water tanks and changing the sink taps [15, 17, 18]. The *C. meningosepticum* outbreak in our study was only controlled by reinforcement of the usual measures and emphasis on routine hand hygiene among staff. Our findings, hence, indicate that *C. meningosepticum* neonatal colonization outbreaks may not proceed to infection and that minor outbreaks may be successfully managed with reinforcement of the standard precautions.

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