JOURNAL OF CLINICAL MICROBIOLOGY, May 1995, p. 1426–1427 Vol. 33, No. 5
0095-1137/95/$04.00+0

Evaluation of Novel Vancomycin-Containing Medium for Primary Isolation of *Kingella kingae* from Upper Respiratory Tract Specimens

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Received 3 January 1995/Returned for modification 26 January 1995/Accepted 21 February 1995

A new selective medium (BAV), consisting of trypticase agar with 5% sheep hemoglobin and 2 μg of vancomycin per ml, was compared with the routine blood-agar medium for the primary isolation of *Kingella kingae* from upper respiratory specimens from a population of young children. Infection was detected by the BAV medium in 43 of 44 (98%) cultures positive for *K. kingae*, and detection of the organism was facilitated by inhibition of gram-positive flora. Infection was detected in only 10 of 44 (23%) positive cultures by the blood-agar medium, and plates were usually covered by abundant normal flora, making the recognition of *K. kingae* much more difficult. Challenge of the medium with different organisms of respiratory origin showed that the BAV medium was inhibitory for gram-positive cocci and *Haemophilus influenzae* but that it supported growth of eight *K. kingae* strains isolated from patients with invasive infections. The new medium appears to be a useful epidemiological tool for studying the respiratory carriage of *K. kingae*.

In recent years, *Kingella kingae*, a hemolytic gram-negative bacillus of the family *Neisseriaceae*, has been increasingly recognized as an important cause of invasive infections in young children, especially of those involving the skeletal system (2, 3, 9, 10). Despite this renewed interest in the organism, once considered an exceptional cause of human disease, the epidemiology of *K. kingae* remains incompletely understood. *K. kingae* has been recovered from 1% of respiratory cultures, but this figure can be considered only a minimal estimate (4). Because of the slow growth of the organism compared with other members of the normal respiratory flora which tend to cover the agar surface, without a selective medium, recognition of *K. kingae* in nasopharyngeal cultures is difficult and often impossible. Although Thayer-Martin and other selective media specifically designed for members of the *Neisseriaceae* support the growth of *K. kingae*, in the absence of a specific marker for the organism, its presence in a plate crowded with commensal *Neisseria* strains is frequently inconspicuous.

In an attempt to improve the detection of *K. kingae* in respiratory cultures a novel medium was designed. This medium (BAV) comprises (per liter): casein peptone, 15 g; soy peptone, 5 g; natrium chloride, 5 g; agar, 15 g; yeast extract, 1.75 g; vancomycin, 2 mg; and defibrinated sheep blood, 5 to 7%. It was assumed that hemoglobin-containing medium would facilitate the recognition of this beta-hemolytic organism, whereas the presence of a glycopeptide antimicrobial drug would inhibit growth of the competing gram-positive flora of the respiratory tract.

Although *K. kingae*, being a gram-negative organism, is considered to be resistant to glycopeptide antimicrobial agents, there is only a small amount of data available in the literature supporting this assumption (5, 7). It should also be kept in mind that certain strains of *Neisseria gonorrhoeae*, a related organism belonging to the same family, may be susceptible to vancomycin concentrations of 4 μg/ml (6). Therefore, the susceptibility to vancomycin of eight different *K. kingae* strains, isolated from synovial fluid or blood from children with invasive infections, was studied by the disk diffusion method of Bauer and Kirby and the E test (PDM Epsilometer; AB Biodisk, Solna, Sweden) (1, 9, 10). Although the best medium to determine the antimicrobial susceptibility of *K. kingae* remains to be defined, the organism may fail to grow on unsupplemented Mueller-Hinton agar but it grows well on trypticase agar medium containing 5% sheep blood (BA), and therefore, this medium was chosen for the performance of susceptibility tests (5). After 24 and 48 h of incubation no inhibition zone was observed around the vancomycin disk and the MICs were ≥256 μg/ml for all strains.

In addition, the selectivity of the BAV medium was challenged with members of the respiratory flora. The above-mentioned *K. kingae* isolates as well as one strain each of the species *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Haemophilus influenzae* (untypeable), *Streptococcus sanguis*, and *Staphylococcus aureus* were suspended in 0.9% natrium chloride solution and adjusted to a turbidity equivalent to a 0.5 McFarland standard, plated onto BAV, and incubated at 35°C. All eight *K. kingae* strains and the *M. catarrhalis* strain grew after 24 h, whereas growth of the other organisms was totally inhibited.

To investigate the quantitative recovery of *K. kingae* in the BAV medium, three strains of the organism were grown overnight and suspended in 0.9% natrium chloride. The suspension was serially diluted and plated onto BAV and BA plates. After 48 h of incubation no significant differences in the colony counts were found between the two media.

Finally, the performance of the BAV medium in the detection of *K. kingae* from the pharynx for a population of infants and young children was prospectively examined. Throat and nasopharyngeal cultures were obtained on cotton swabs. Swabs were sent within 1 h to the Clinical Microbiology Laboratory of the Soroka Medical Center, Beer-Sheva, Israel, on modified Stewart medium (Medical Wire & Equipment, Corsham, Wiltshire, United Kingdom). On arrival, swabs were plated in random order on routine BA medium and on BAV plates.

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Both BAV and BA plates were incubated aerobically at 35°C for 48 h. *K. kingae* was identified on the basis of a typical Gram stain, beta-hemolysis, a positive oxidase reaction, a negative catalase reaction, urease and motility tests, and acidification of glucose and maltose but not of other sugars (8). The performance of the BA medium in the detection of *K. kingae* was compared with that of the BAV medium. A test for matched-pair analysis of discrete data (McNemar's test with correction for continuity) was used to assess the statistical significance of the observed differences between discordant BA-BAV pairs.

Overall, *K. kingae* was recovered from 44 pharyngeal cultures. Usually, fewer than 10 colonies of *K. kingae* were detected in positive plates. In general terms, after 48 h BA plates were covered with a mixture of gram-positive and gram-negative organisms, whereas BAV plates were less crowded and most of the growing flora consisted of nonhemolytic strains of *Neisseriaceae*. Although the presence of *K. kingae* could be suspected in some cases after 24 h of incubation, usually after 48 h colonies were developed enough to allow their easy recognition. The performance of the BA and BAV media may be summarized as follows. *K. kingae* was detected by BA and BAV in 9 of the 44 cultures, it was detected by BA only in 1 of the cultures, and it was detected by BAV only in 34 of the cultures ($P < 0.001$). Overall, the BA medium detected *K. kingae* in 10 of the 44 positive cultures and the BAV medium detected it in 43 of the 44 (sensitivities of 23 and 98%, respectively).

It is concluded that the BAV medium supports growth of *K. kingae*, facilitates recognition of the organism by inhibiting growth of competing respiratory flora, and is significantly superior to the routine BA for the primary isolation of *K. kingae* from respiratory tract cultures. It appears that the BAV medium is a convenient epidemiological tool for studying the prevalence of *K. kingae* in the respiratory tract.

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