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Cephalexin-Supplemented Jones-Kendrick Charcoal Agar for Selective Isolation of *Bordetella pertussis*: Comparison with Previously Described Media

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Four agar media, Jones-Kendrick (JK) charcoal agar unsupplemented, JK agar supplemented with 0.5 U of penicillin per ml, JK medium supplemented with 2.5 μg of methicillin per ml, and JK medium supplemented with 40 μg of cephalexin per ml, were evaluated to determine their ability to support growth of *Bordetella pertussis*, their ability to selectively inhibit normal pharyngeal flora while maintaining growth of *B. pertussis*, and their stability during storage. Five stock cultures of *B. pertussis* were plated on each of the media. Penicillin- and cephalexin-supplemented media were more inhibitory for early growth of *B. pertussis* than was medium supplemented with methicillin. However, after 5 days of incubation at 35°C, all media supported good growth of this organism. When employed to detect *B. pertussis* in sham specimens, prepared by mixing normal pharyngeal material with each of the five *B. pertussis* stock cultures, the medium containing cephalexin was judged superior to all other media tested in its combined ability to suppress the growth of normal pharyngeal flora and to allow early detection of *Bordetella* colonies. All media tested retained their efficacy after 9 weeks of storage at 2 to 8°C.

Refinements in media designed for the isolation of *Bordetella pertussis* have been sought since Bordet-Gengou (BG) medium was first described (1). Although the original medium excluded peptone and was adjusted to a pH of 5.0 to inhibit the growth of normal pharyngeal flora (NPF), BG medium was essentially nonselective, and overgrowth of *B. pertussis* was common. With the advent of penicillin, Fleming demonstrated that this substance could be used for the selective culture of *B. pertussis* (4). This antibiotic has since been used successfully in diagnostic BG plates (6).

Because of the difficulty of preparation and the restricted shelf-life, blood-free media were developed (3, 8, 10). Fatty acids toxic to *B. pertussis* are adsorbed by charcoal rather than through the addition of blood. Jones and Kendrick modified the medium of Mishulow and included penicillin at a concentration of 0.3 U/ml (5). This semiselective medium (JK medium) is blood free, has a shelf-life of 2 to 3 months, and may be used both for transport and subsequent culture of *B. pertussis* (9).

Although the addition of penicillin to either BG or charcoal medium does aid in the isolation of *B. pertussis* by inhibiting the growth of NPF, selectivity is partial, and overgrowth remains an important problem in the isolation of *B. pertussis*. Using BG medium, Broom and colleagues compared penicillin and methicillin and found methicillin to be a superior selective agent (2). Earlier, Sutcliffe and Abbott studied a charcoal medium similar to that described by Jones and Kendrick. This medium was additionally supplemented with blood and was used to compare penicillin (0.25 U/ml) with cephalexin (40 μg/ml); cephalexin was found to be superior (12).

Although Regan and Lowe have shown that a half-strength modification of the medium described by Sutcliffe and Abbott will serve very well as a transport medium for *B. pertussis* (11), many laboratories employ JK medium for both transport and culture. Using JK medium, we evaluated penicillin, methicillin, and cephalexin supplementation in the isolation of *B. pertussis*. Media were compared for ability to support the growth of this organism, ability to suppress the growth of admixtures of NPF, and stability during storage.

MATERIALS AND METHODS

Isolation media. Four agar media, JK agar unsupplemented, JK agar supplemented with 0.5 U of penicillin per ml, JK agar supplemented with 2.5 μg of methicillin per ml, and JK agar supplemented with 40 μg of

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TABLE 1. Comparison of *B. pertussis* growth on unsupplemented JK medium and on JK medium supplemented with penicillin, methicillin, or cephalexin[a]

B. pertussis inoculum	JK medium	Growth on indicated days:														
		Strain 5373			Strain 116			Strain 106			Strain 105			Strain 560		
		1	3	5	1	3	5	1	3	5	1	3	5	1	3	5
Pure culture	Plain	0	1	4	0	2	4	0	2	4	0	2	4	0	1	4
	0.5 U of penicillin per ml	0	+/−	2	0	+/−	2	0	+/−	2	0	1	3	0	1	2
	2.5 μg of methicillin per ml	0	1	4	0	2	4	0	2	4	0	2	4	0	2	4
	40 μg of cephalexin per ml	0	1	3	0	1	3	0	1	3	0	1	3	0	1	4
Oral secretions mixture[b]	Plain	0	0	1	0	0	2	0	+/−	3	0	+/−	3	0	0	1
	0.5 U of penicillin per ml	0	0	2	0	0	+/−	0	+/−	2	0	+/−	2	0	1	2
	2.5 μg of methicillin per ml	0	1	3	0	+/−	3	0	1	4	0	1	4	0	1	4
	40 μg of cephalexin per ml	0	+/−	3	0	+/−	3	0	1	3	0	1	3	0	1	4

[a] Growth was recorded as 0 for no growth and +/− through 4+ for barely visible to mature colony development.

[b] Recorded values for mixtures represent *B. pertussis* colony development only and do not document the relative amounts of *B. pertussis* versus oral secretions present.

cephalexin per ml, were evaluated. All media were dispensed into 100-mm sterile plastic petri dishes (15 to 20 ml/plate), sealed in plastic to prevent dehydration, and stored at 2 to 8°C before use.

Inoculum and incubation. Five *B. pertussis* isolates (no. 105, 560, 116, 106, and 5373), provided by the Centers for Disease Control, Atlanta, Ga., were maintained by weekly subcultures on JK medium containing no antibiotics. Fresh subcultures (36 to 72 h) were used to prepare pure culture saline suspensions equivalent to a 0.5 McFarland turbidimetric standard. Each *B. pertussis*-NPF mixture was prepared by adding to the previously prepared *B. pertussis* suspensions an equal volume of oral secretion adjusted with saline to a 0.5 McFarland standard.

All media being evaluated were tested during the first week after preparation and after 9 weeks of storage at 2 to 8°C. Plates were inoculated with each pure culture *B. pertussis* suspension and each *B. pertussis*-NPF mixed suspension by transferring 0.001 and 0.002 ml, respectively, to the agar surface. Inocula were then dilution streaked into each of four quadrants, flaming the loop between quadrants 2, 3, and 4. All plates were incubated at 35°C in a candle extinction jar and observed daily for a total of 5 days.

TABLE 2. Comparison of NPF growth on unsupplemented JK medium and JK medium supplemented with penicillin, methicillin, or cephalexin[a]

JK medium	Growth of NPF on day:		
	1	3	5
Plain	4	4	4
0.5 U of penicillin per ml	1	3	3
2.5 μg of methicillin per ml	2	3	3
40 μg of cephalexin per ml	0	0	1

[a] See footnote *a* of Table 1 for explanation of values.

RESULTS

JK agar unsupplemented and JK agar supplemented with penicillin, methicillin, or cephalexin were compared for their ability to support the growth of *B. pertussis* and to inhibit the growth of NPF. As seen in Table 1, each medium tested supported the growth of *B. pertussis*. Media containing either penicillin or cephalexin appeared to be slightly more inhibitory for the growth of this organism in pure culture than did either medium containing methicillin or medium without antibiotic. However, after 5 days of incubation at 35°C, all media supported good growth of all strains tested.

When compared with JK medium without antibiotics, medium containing cephalexin was consistently more inhibitory for NPF throughout the incubation period than was medium containing penicillin or methicillin (Table 2). This resulted in better overall growth of *B. pertussis* colonies and greater ease in detection (Fig. 1).

After 9 weeks of storage at 2 to 8°C, there appeared to be a slight reduction in the ability of each medium to suppress the growth of NPF and the ability to support the growth of *B. pertussis*. However, for medium containing cephalexin, this reduction in the suppression of NPF was insignificant and did not adversely affect its use after storage.

DISCUSSION

Although many media have been described in the literature for the transportation and isolation of *B. pertussis*, JK charcoal agar remains a standard for use either alone or in conjunction with BG agar. Penicillin, methicillin, and cephalexin have each been studied and evaluated for use in the preparation of a semiselective medi-

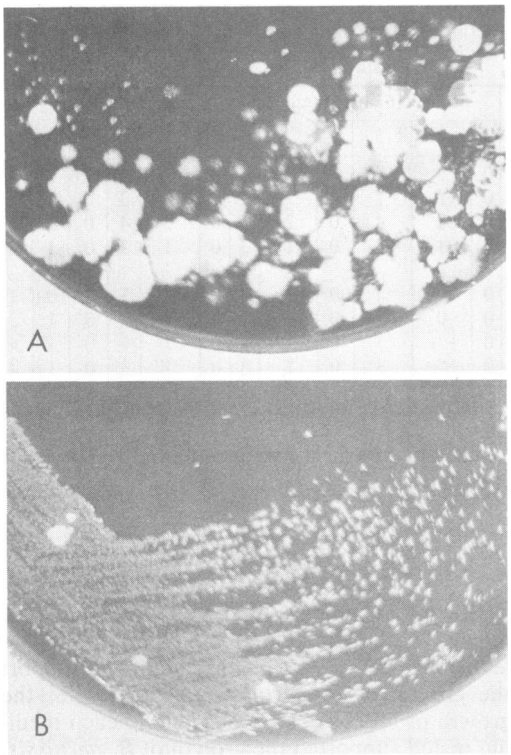

FIG. 1. (A) *B. pertussis*-NPF mixture on unsupplemented JK medium. (B) *B. pertussis*-NPF mixture on JK medium supplemented with cephalexin, 40 μg/ml. Plates were incubated for 5 days at 35°C.

um; however, to our knowledge, there has been no single study which used JK agar and compared all three antibiotics simultaneously. The concentrations chosen for the antibiotics in this study were those suggested in the literature (2, 9, 12).

As seen in the data presented in Table 1, both penicillin- and cephalexin-supplemented media were slightly more inhibitory for the growth of *B. pertussis* than was the medium containing methicillin or the antibiotic-free medium. With regard to cephalexin, this was more than compensated for by its increased ability to suppress the growth of NPF (Table 2), since the presence of NPF has an inhibitory effect on the growth of *B. pertussis* (7). Although methicillin was less inhibitory for the growth of this organism than either penicillin or cephalexin, it was also less inhibitory for the growth of NPF when compared with cephalexin. It may not be obvious from the data in Table 1 for *B. pertussis*-oral secretions that cephalexin is the better supplement. The data reflect the presence of *B. pertussis* colonies and the degree of colony development. The total number of colonies present and the ease of detection are not recorded (Fig. 1).

After considering all parameters, it is our opinion that the antibiotic-free medium was of little use in the isolation of *B. pertussis* when mixed with oral secretions. JK medium containing penicillin was only slightly better than medium containing no antibiotic. Methicillin was superior to penicillin as a selective ingredient. However, cephalexin was the antibiotic of choice. The ultimate test of this medium will require the careful review of its field use. We have employed the medium in the field for sporadic cases of pertussis and have found it useful.

As mentioned, the media used in this study were wrapped in plastic to preserve the original moisture content. After 2 months of storage at 2 to 8°C, JK medium with cephalexin retained essentially all of its original efficacy. Because of this stability, we have found it convenient to maintain a supply of both JK agar slants for transport of specimens to the laboratory and JK agar plates for subsequent inoculation in the laboratory upon receipt of the transport tube specimen.

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