Technical methods

Experiences in the use of commercial antisera for the capsular typing of klebsiella species

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Following the work of Julianelle (1926), Kauffman (1949), Brooke (1951), Worful and Ferguson (1959), Edwards and Fife (1952, 1955), Edmunds (1954), and Orskov (1955) the klebsiellae are divisible into 72 capsular (K) types. Little is known in this country about the epidemiology of klebsiella (*Lancet*, 1971). Work is currently hindered by there being no centre for capsular typing, presumably due to the difficulty of rearing 72 absorbed specific antisera. However, these sera have recently been made available com-

mercially (Difco).

This paper describes a technique for detecting agglutination and capsular swelling reactions of klebsiella, the assessment of the commercial antisera, and the use of these sera for determining the capsular types of unknown strains.

Preparation of Suspensions

A single colony of klebsiella from a fresh blood agar or MacConkey agar culture is subcultured onto a 15 ml Worfel-Ferguson agar (Difco 0924) plate and incubated at room temperature (18-24°C) for 36 hours.

Large amounts of culture are harvested with a large triangular loop (1 cm wide, SWG 22) and added to a custom-built homogenizer (Fig. 1) containing 3 ml of 10% formol phosphate-buffered saline pH 7.3. A few movements of the plunger quickly homogenizes even the most mucoid culture. The final heavy suspension should have a milky opacity somewhat greater than Brown's opacity

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Fig. 1 Custom-built homogenizer for preparing formol saline suspension.

tube no. 10 (approximately 10^{10} organisms/ml). This suspension is suitable for slide agglutination reactions.

A more dilute formal saline suspension is then made for capsular swelling reactions, equivalent to Brown's tube no. 3 (approximately 10^9 organisms/ml).

Examination for Capsules

A loopful (4 mm diameter, SWG 24) of heavy suspension is placed on a 76 mm $\times$ 50 mm microscope slide adjacent to an equal volume of Nigrosin and the 2 drops run together under a coverslip. Six strains can be examined on one slide. Ordinary light microscopy using a $\times 100$ objective and reduced substage illumination displays the capsules, the width of which are usually one third to half the diameter of the bacillus. Cultures yielding suspensions of noncapsular organisms should be discarded, but under the cultural conditions described this is rarely necessary.

Reconstitution of Sera

One ml of pH 7.0 sterile distilled water is used to

reconstitute each freeze-dried serum. The manufacturer's instructions do not specify the pH to be used.

Slide Agglutination

One small loopful (1.25 mm diameter, SWG 28) of heavy suspension is mixed with a larger loopful (2.25 mm diameter, SWG 28) of serum on a glass slide. A positive agglutination reaction usually appears quickly and is coarsely granular. More mucoid strains produce an immiscible scum. Using these cultural conditions a few strains may display positive reactions which appear slowly, are finely granular, and may not be visible to the naked eye. A hand lens should be used to examine all slide agglutination reactions.

Capsular Swelling (Quellung Reaction)

One 2.25 mm diameter loopful of dilute suspension is mixed with an equal volume of undiluted serum. After two or three minutes the preparation is examined under a coverslip using phase contrast microscopy with a $\times 100$ oil immersion objective. A negative control consisting of suspension only is

Fig. 2a *Klebsiella capsular (K)* strain 30 with specific serum 31. Capsules appear as ill defined halos (phase contrast $\times 1\,850$).

Fig. 2b Strain 30 with specific serum 30 showing agglutination and peripheral delineation of capsules (phase contrast $\times 1\,850$).

similarly examined.

NEGATIVE RESULTS

The dark grey bacilli appear singly, float freely in the suspension, exhibit Brownian movement, and may be sharply focused (Fig. 2a). Some slight white 'haloing' may be evident in the area adjacent to the bacillus, but the peripheral edges of the capsules are indistinct. Extracapsular slime is not visible.

POSITIVE RESULTS

The bacilli are immobilized in agglutinates and are difficult to focus sharply. The capsules are conspicuous, may appear 'swollen' and have a thin black line delineating their peripheral margin (Fig. 2b). If extracapsular slime material is present it appears as strands of finely granular, structureless material which enmeshes the bacilli (Fig. 3b).

Assessment of Commercial Antisera

The 18 pooled sera and 72 specific sera were tested

for agglutination and capsular swelling reactions against known type strains supplied by Dr Ida Orskov. Known serotypes were tested with between three to 39 heterologous sera to detect non-specific cross reactions.

POOLED ANTISERA

Table I shows the agglutination and capsular swelling reaction results when the 18 pools were tested against homologous type strains. Pools failing to produce capsular swelling reactions failed also to give a positive slide agglutination.

SPECIFIC ANTISERA

Specific sera 43 and 27 produced neither positive agglutination nor capsular swelling reactions, but all other specific sera produced both positive agglutination and capsular swelling when tested against known serotypes.

Non-specific agglutination reactions were rarely found under these cultural conditions, but with suspensions derived from media relatively rich in

Fig. 3a Strain 25 with specific serum 24. Slime material not visible (phase contrast $\times 1850$).

Fig. 3b Strain 25 with specific serum 25. Refractile slime and conspicuous capsules (phase contrast $\times 1850$).

Pool Number	Containing Specific Antisera	Known Capsular (K) Strains Giving Capsular Swelling and Agglutination with Pool	Known Capsular (K) Strains Failing to Give Capsular Swelling and Agglutination with Pool
1	1, 2, 3, 24	1, 2, 3, 24	—
2	4, 5, 6, 7	4, 5, 6, 7	—
3	8, 9, 10, 25	8, 9, 10, 25	—
4	13, 14, 15, 16	13, —, 15, 16	14
5	17, 18, 19, 20	—, 18, —, 20	17, 19
6	21, 22, 23, 11	21, 22, 23, 11	—
7	26, 27, 28, 30	26, 27, 28, —	30
8	12, 29, 40, 41	12, —, 40, 41	29
9	31, 32, 43, 44	—, 32, 43, 44	31
10	33, 34, 35, 36	—, 34, 35, —	33, 36
11	37, 38, 39, 42	37, 38, —, 42	39
12	45, 46, 47	45, 46, 47	—
13	48, 49, 50, 51	48, 49, 50, —	51
14	52, 53, 54, 55	52, 53, 54, —	55
15	56, 57, 58, 59	56, 57, —, 59	58
16	60, 61, 62, 63	60, 61, 62, 63	—
17	64, 65, 66, 67, 68, 69	64, 65, 66, 67, 68, 69	—
18	70, 71, 72	70, 71, 72	—

Table I Results of testing known capsular (K) strains against pooled sera

nitrogen non-specific agglutinations occurred with strains 14, 16, 17, 29, 41, 32, 45, 58, 59, 62, 66, 69, and 70.

Only one capsular swelling cross reaction has been demonstrated with the specific sera in the examination of 1 207 of the 5 112 possible cross reactions. Specific serum 53 reacts with both 53 and 47 type strains.

Typing Unknown Strains

Because of the failure of some pools it was necessary to include specific sera 14, 17, 19, 30, 29, 31, 33, 36, 39, 51, and 58 with pools 1 to 18 to give a complete set of screening sera.

In view of the difficulties occasionally encountered with weak or non-specific slide agglutination reaction this technique has been abandoned for typing unknown strains.

Weak suspensions of the unknown strains are tested against all the screening sera for capsular swelling. Where a positive result occurs with a pool, the strain is tested for capsular swelling with the specific sera included in that pool.

Comments

With experience, microscopical examination for capsular swelling takes a few seconds for each reaction and is faster than slide agglutinations.

Positive reactions are easily detected using this technique.

The reason for the failure of some pools is not clear, especially as the specific sera work so well. A preliminary examination of pools with different batch numbers has yielded the same results. The problem has been taken up with the manufacturers and it is hoped that the pools will be improved in the near future.

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References

- Brooke, M. S. (1951). Further capsular antigens of klebsiella strains. *Acta path. microbiol. scand.*, **28**, 313-327.
- Edmunds, P. N. (1954). Further klebsiella capsule types. *J. infect. Dis.*, **94**, 65-71.
- Edwards, P. R., and Fife, M. A. (1952). Capsule types of klebsiella. *J. infect. Dis.*, **91**, 92-104.
- Edwards, P. R., and Fife, M. A. (1955). Studies on the klebsiella—aerobacter group of bacteria. *J. Bact.*, **70**, 382-390.
- Julianelle, L. A. (1926). A biological classification of *Enkapsulatus pneumoniae* (Friedländer's bacillus). *J. exp. Med.*, **44**, 113-128.
- Kauffman, N. F. (1949). On the serology of the klebsiella group. *Acta path. microbiol. scand.*, **26**, 381-406.
- Lancet (1971). Epidemiology of klebsiella infections. (Leading article). *Lancet*, **2**, 416-417.
- Orskov, I. (1955). Serological investigations in the klebsiella group. I. New capsule types. *Acta path. microbiol. scand.*, **36**, 449-453.