# An investigation into the incidence and sources of klebsiella infections in hospital patients

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## SUMMARY

Coliforms isolated from infections over a period of 22 months were examined and 16% were shown to be klebsiellas. The biochemical reactions and serotypes of these klebsiellas were determined and the relationship between species, serotype and type of infection investigated. Although no obvious outbreaks of infection occurred during this period a number of clusters of isolations of the same serotype of klebsiella were found.

Using an enrichment method for the isolation of klebsiellas from faeces, sero and bacteriocin typing, and the examination of ten colonies, it was demonstrated that half of the patients carried a klebsiella of the same type in the bowel as caused the infection.

## INTRODUCTION

In recent years there have been numerous reports of outbreaks of infection caused by multiply antibiotic-resistant klebsiellas (Price & Sleigh, 1970; Martin *et al.* 1971; Curie *et al.* 1978) but there is not a great deal of information about the normal incidence of klebsiella infections in a general hospital. Little is known about the sources and modes of spread of these organisms although Selden *et al.* (1970) provided evidence that the patient's own bowel may be an important source of the infecting organism, as is thought to be the case with other gram-negative bacilli (Darrell & Wahba, 1964; Grüneberg, Leigh & Brumfitt, 1968).

For these reasons we have examined all coliform bacilli isolated in the diagnostic laboratory here to determine the incidence of klebsiella infections and have attempted to assess the relationship between colonization of the gastro-intestinal tract with klebsiellas and subsequent development of infection. We have also made a comparative study of methods for the isolation of klebsiellas from faeces in order to obtain the highest possible isolation rate.

## MATERIALS AND METHODS

*Identification of klebsiellas causing infections in hospital patients*

All coliform bacilli isolated in the diagnostic laboratory were examined to determine if they were klebsiellas by inoculation into a screening medium (Donovan, 1966) and Christensen's urea medium. Non-motile, hydrogen sulphide

negative, inositol fermenting and urea hydrolysing organisms were further identified by the methods of Cowan & Steel (1974) into the following species, *K. aerogenes*, *K. pneumoniae*, *K. atlantae*, *K. edwardsii*, *K. ozaenae* and *K. rhinoscleromatis*. A total of 3173 coliforms were examined over a period of 22 months.

*Isolation of klebsiellas from faeces*

During part of this survey, faecal samples were requested from all patients with klebsiella infections and these were examined for the presence of klebsiellas. Fifty-five specimens of faeces were obtained from these patients.

The method used for isolating klebsiellas from faeces was developed after a comparative study of a variety of methods with different combinations of media. For this study faeces from 56 hospital patients were examined; they were obtained from female orthopaedic patients and from hospital patients who were known to have klebsiella infections. Either the media were inoculated directly with a swab of faeces or 1 g faeces was emulsified in 2 ml of $\frac{1}{4}$ strength Ringer's solution and a swab of the suspension was used to inoculate the media.

The solid media used were: deoxycholate agar (Oxoid), Simmon's citrate agar (Difco), MacConkey agar (Oxoid) and MacConkey inositol carbenicillin agar (MIC agar), which contains sodium taurocholate 5 g, peptone 20 g, inositol 10 g, agar 15 g, 5 ml of a 1 % aqueous solution of neutral red, distilled water 1 litre and 10 $\mu$g/ml of carbenicillin.

MIC agar is a modification of the medium described by Thom (1970), but with 10 $\mu$g/ml of carbenicillin instead of 100 $\mu$g/ml. This lower concentration of carbenicillin was used as it was found that of 50 klebsiella strains recently isolated in the routine diagnostic laboratory, 48 could be cultured at this concentration but only 44 could be cultured if 100 $\mu$g/ml of carbenicillin was used. Of 50 lactose-fermenting coliforms, which were not klebsiellas, 10 could be cultured at 10 $\mu$g/ml of carbenicillin and 6 at 100 $\mu$g/ml. Klebsiella colonies on MIC agar are typically large and pink, much the same as their appearance on MacConkey agar.

Selenite broth (Difco) and Koser's citrate broth (Difco) were also used and subcultures were made to solid media after different periods of incubation.

As a result of the comparative study (see Results), the following method was chosen as giving the highest isolation rate of klebsiellas: a specimen of faeces was inoculated with a swab to MacConkey agar, MIC agar and citrate agar, and into citrate broth. After incubation at 37 °C for 24 h the citrate broth was subcultured to MacConkey, MIC and citrate agar. If no klebsiellas were isolated, the citrate broth was again subcultured to the same three media after 48 h, and was also inoculated into another citrate broth which was subcultured after a further 48 h incubation.

*Serotyping*

All infecting strains and strains from faeces were serotyped by the quellung method (Kauffmann, 1949) using 77 capsular antisera produced in this laboratory.

Table 1. *Table to show the number of specimens out of a total of 56 from which klebsiellas were isolated*

| Media | Method of inoculation | | Media | Method of inoculation | |
|---|---|---|---|---|---|
| | Swab of faeces | Suspension of faeces | | Swab of faeces | Suspension of faeces |
| 1 | 15 | 16 | 9 | 27 | 25 |
| 2 | 25 | 25 | 10 | 24 | 24 |
| 3 | 14 | 16 | 11 | 31 | 28 |
| 4 | 23 | 23 | 12 | 27 | 26 |
| 5 | 16 | 16 | 13 | 29 | 27 |
| 6 | 12 | 12 | 14 | 30 | 29 |
| 7 | 22 | 19 | 15 | 30 | 30 |
| 8 | 29 | 27 | | | |

1 = MacConkey agar
2 = MacConkey inositol carbenicillin agar
3 = Deoxycholate agar
4 = Citrate agar
5 = Selenite broth subcultured to MacConkey agar
6 = Selenite broth subcultured to MacConkey inositol carbenicillin agar
7 = Citrate broth subcultured at 24 h to MacConkey agar
8 = Citrate broth subcultured at 24 h to MacConkey inositol carbenicillin agar
9 = Citrate broth subcultured at 24 h to citrate agar
10 = Citrate broth subcultured at 48 h to MacConkey agar
11 = Citrate broth subcultured at 48 h to MacConkey inositol carbenicillin agar
12 = Citrate broth subcultured at 48 h to citrate agar
13 = Citrate broth subcultured at 96 h to MacConkey agar
14 = Citrate broth subcultured at 96 h to MacConkey inositol carbenicillin agar
15 = Citrate broth subcultured at 96 h to citrate agar

*Homogeneity of serotypes in specimens from infections and faecal specimens*

Ten klebsiella colonies were picked from culture plates of 33 specimens from infections and 36 faecal specimens, and were serotyped to determine whether the colonies in any one set were all of the same serotype.

## RESULTS

*Comparison of methods for isolation of klebsiellas from faeces*

Fifteen methods for the isolation of klebsiellas from faeces were compared, using different combinations of media and subculturing after different periods of incubation. The same 56 specimens of faeces were examined by each method and specimens were inoculated onto media by a swab and from a suspension. The number of klebsiellas isolated from the 56 faecal specimens by each method is shown in Table 1. The solid media giving the highest direct isolation rates were MIC agar and citrate agar. The highest isolation rates were obtained by subculturing citrate broth to MIC agar and citrate agar. Inoculation of media by means of a suspension of faeces did not give a higher isolation rate than inoculation by a swab.

Table 2. *The incidence of klebsiellas in different types of infection*

|  | Urine | Swabs | Sputum | Others* |
|---|---|---|---|---|
| Number of coliform infections | 2006 | 746 | 283 | 138 |
| Number of klebsiella infections | 250 | 132 | 87 | 32 |
| Percentage of klebsiella infections | 12·5 | 17·7 | 30·7 | 23·2 |

\* Blood cultures, cerebro-spinal fluids, catheter tips and other sources.

Table 3. *Sources and serotypes of strains identified as Klebsiella species other than* K. aerogenes

| Species | Source | Capsular serotype |
|---|---|---|
| K. pneumoniae | Sputum | 12 |
| K. pneumoniae | Sputum | 32 |
| K. pneumoniae | Wound swab | 12 |
| K. pneumoniae | Wound swab | 19 |
| K. pneumoniae | Urine | 21 |
| K. pneumoniae | Catheter tip | 32 |
| K. edwardsii | C.S.F. | NT |
| K. edwardsii | C.S.F. | NT |
| K. edwardsii | Urine | 66 |
| K. edwardsii | Urine | G23 |
| K. ozaenae | Urine | G23 |

NT, non typable. G23 may be a new serotype related to type 57.

*Incidence of klebsiella infections*

Five hundred and one (16 %) of the 3173 coliform infections examined were caused by klebsiellas. The incidence of klebsiellas in different types of infection is shown in Table 2.

Of the 501 klebsiellas causing infections, 490 (98 %) were identified as *K. aerogenes*. The remainder were identified by their biochemical reactions as other species of *Klebsiella*, and the species, sources and serotypes of these 11 strains are given in Table 3.

*Distribution of capsular serotypes in the infecting strains*

Four hundred and twenty-four (84·6 %) isolates were typable serologically and all but 15 of the 77 capsular serotypes of klebsiellas were found on at least one occasion. The most common type found, type 35, was represented by 27 strains, and twelve other serotypes were isolated on ten or more occasions; these were types 2, 7, 8, 12, 15, 16, 18, 33, 38, 43, 47, and 62. There was no apparent relationship between any particular serotype and a particular type of infection.

There was little evidence that outbreaks of klebsiella infection had occurred during this study although there were a number of occasions when a few klebsiella strains of the same serotype were isolated from patients on the same ward over a short period. The largest cluster was a group of five strains of serotype 16 isolated from a variety of sources from patients on the same ward over a period of a month.

In addition seven pairs of isolates of the same serotype were obtained within 3 days of each other from patients on the same ward, and were considered to be related.

*Isolations of klebsiellas from faeces*

Fifty-five specimens of faeces were examined and 45 (81·8 %) contained klebsiellas. The same serotype was present in the faeces as in the infection in 21 patients (38·2 %), in 5 patients (9·1 %) faeces and infection both contained strains of klebsiellas which were non-typable and 19 faecal specimens (34·5 %) contained a different serotype of klebsiella from that present in the infection. Bacteriocin typing (Edmondson and Cooke, 1979) revealed similarities between the pairs of serologically non-typable isolates from two patients. Neither the site of infection, nor the time interval between isolation of klebsiellas from the infection and receipt of the faecal specimen affected these results.

*Homogeneity of serotypes in klebsiella cultures from infections*

Ten klebsiella colonies were examined from 33 specimens from infections and in 28 all ten colonies were of the same serotype. Of the five which showed differences, three had nine identical colonies and one different, and the remaining two had approximately equal numbers of typable and non-typable strains. Four of the five heterogeneous sets of colonies were isolated from sputum and one from a catheter tip.

*Homogeneity of serotypes in klebsiella cultures from faeces*

Thirty-six specimens of faeces were examined and in 27 the ten colonies were the same serotype. Of the nine which showed differences five had only one colony different, two contained three serotypes and two contained five serotypes.

In 29 patients ten colonies were examined from both the specimen from the infection and the faecal sample. In three cases a similarity was detected by typing ten colonies which would not have been detected by examination of the first colony from each source. In two instances the similarity was detected by the later isolation of a strain from the faecal specimen that was the same serotype as the infecting strain, and on one occasion by the isolation from the infection of a strain similar to the first colony isolated from faeces.

## DISCUSSION

In this paper we report the incidence of klebsiella infections in a general hospital over a period of 22 months, during which time no outbreaks of infection occurred. Klebsiella infections comprised 16 % of the coliform infections reported in the diagnostic laboratory. Coliform infections were considered to be infections caused by organisms giving rise to lactose-fermenting colonies on MacConkey agar and many of the klebsiellas isolated were indistinguishable by their colonial appearance from other coliforms. These cultures were not mucoid and would not have been suspected of being klebsiellas without examination of their biochemical properties.

In this hospital coliforms comprise approximately 34 % of the total organisms

causing infection, so that klebsiellas are responsible for approximately 5 % of the total infections reported in the diagnostic laboratory.

The great majority of the infections (98 %) were caused by *K. aerogenes*, but it is interesting that 11 infections were due to other species of *Klebsiella* and that these were not always isolated from the respiratory tract. The isolation of strains with the biochemical properties of *K. pneumoniae*, *K. edwardsii* and *K. ozaenae* from specimens of urine is unexpected and the serotypes are not those which are generally considered to be associated with these species. The correlation between these species and serotype as given by Cowan & Steel (1974) is that *K. pneumoniae* is type 3, *K. edwardsii* may be either type 1 or 2, and *K. ozaenae* may be types 4, 5, or 6. Twenty-three of the 501 strains isolated were of types 1–6, but all had biochemical reactions typical of *K. aerogenes*. There have been few reports of strains of *K. pneumoniae*, *K. edwardsii* and *K. ozaenae* being of serotypes other than 1–6, although Durlakowa, Lachowicz and Slopek (1967) recorded strains of these species of various higher serotypes.

Although the number of *Klebsiella* species other than *K. aerogenes* examined in this study is small, there is a suggestion that the association of species with infections at particular sites of the body and also the association of species with certain serotypes may not be clearly defined.

Most of the specimens examined were urines and from these 12 % of the coliforms were klebsiellas; the incidence in wound infections was higher (18 %) and higher still (31 %) in sputa. The sputa were all purulent specimens giving rise to a heavy or pure growth of the coliform, but we have no evidence as to whether these patients had clinical evidence of chest infection.

There was a broad spread of serotypes among the 501 klebsiella strains and no particular types predominated to any large extent. No clear association was demonstrated between serotype and site of infection although it was perhaps significant that only two of the 23 strains of serotypes 1–6 were isolated from urine while eight were isolated from sputum and 13 from swabs. Previous workers produced evidence that the majority of strains of types 1–6 were isolated from sputum, although other types were just as frequently associated with respiratory disease (Edwards & Fife, 1955).

Serological studies of klebsiella infections in some hospitals have shown the predominance of one or two serotypes and in some cases these types have remained the most common when the surveys were repeated some years later (Ørskov, 1952, 1954; Steinhauer *et al.* 1966; Dans *et al.* 1970; Martin, Yu & Washington, 1971). Other surveys, like this one, have revealed a large number of different types, with no particular types predominating (Davis & Matsen, 1974; Schoutens *et al.*1975). It is clear from these broad epidemiologic studies that the distribution of specific klebsiella serotypes and their association with urinary, respiratory and other infections may vary widely among different hospitals and with time.

There were some indications of clustering of infections but we have no evidence from this work as to whether this was due to patient-to-patient transfer or infection from a common source. We did, however, obtain evidence that intestinal carriage may be important as a source of the infecting organism.

In relating faecal carriage of an organism to the subsequent development of infection it is important that good isolation methods are used to obtain the organism from the faeces where it may be present as a minority population. The use of citrate agar for the isolation of klebsiellas from faeces has been reported by Montgomerie *et al.* (1970), and Thom (1970) used MacConkey inositol carbenicillin agar. Although these media gave good results a higher isolation rate was obtained when a liquid citrate medium was used initially and then subcultured to either citrate or MIC agar. This method allowed klebsiellas to be isolated from the faeces of 81·8 % of patients with a klebsiella infection, and a klebsiella strain of the same serotype was isolated from the faeces and from the infection in 37 % of patients. If one includes the additional similarities shown by klebecin typing serologically non-typable strains and by serotyping ten colonies from each source, then there is evidence that in this type of investigation one can demonstrate faecal carriage of the same serotype in approximately 50 % of patients with infections. Prospective studies would be of value in studying this problem.

The homogeneity of strains from infections was high; faecal strains showed slightly greater heterogeneity and it was of benefit to type ten colonies from this source to prevent similarities between faecal and infecting strains being missed.

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