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# Terminal bronchopneumonia. A bacteriological and histological study of 111 necropsies

By ELIZABETH I. TANNER, J. D. GRAY, P. V. N. REBELLO and D. R. GAMBLE

*Area and Public Health Laboratories, West Park Hospital, Epsom, Surrey*

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Terminal bronchopneumonia usually occurs in patients already debilitated by serious illness. It may be caused by highly virulent bacteria or by opportunistic organisms of low pathogenicity and cases occurring in hospital may be a sensitive indicator of the pathogens present in the hospital environment. In the past 25 years great changes have occurred in the pattern of hospital infection, attributable largely to the introduction of antibiotics. There has been a decline in importance of many familiar pathogens and *Staphylococcus aureus* has become a major problem. More recently there have been indications that the Gram-negative bacilli are becoming more important causes of infection (Finland, Jones & Barnes, 1959; Kneeland & Price, 1960; Lepper, 1963). Some of these bacteria are of low grade pathogenicity but they can produce severe infections in patients with diminished resistance.

This investigation was an attempt to determine the pattern of infection in terminal bronchopneumonia in a series of necropsies.

## MATERIALS AND METHODS

A total of 111 consecutive necropsies in seven mental hospitals, during the months of February to October 1967, were investigated histologically and bacteriologically. The average age of the patients was about 70 years (range 20–92) and the sex distribution was approximately equal. When necropsy was not possible within a few hours of death, the body was refrigerated. All lungs were examined histologically and other organs as indicated by macroscopic appearances.

### *Bacterial isolation and identification*

Swabs for bacteriological investigation were taken from lungs, spleen and colon. The body was opened without disturbing the viscera, a new pair of disposable rubber gloves being worn for each necropsy. With a hand inserted in the pleural cavity the posterior surface of the lung was palpated from apex to base; without withdrawing the hand the lung was lifted enough to permit swabs to be plunged into lung tissue through the untouched anterolateral surface selecting consolidated areas when present. Similarly the spleen was lifted by its pedicle and swabs were pushed through the untouched anterior surface. Faeces were sampled by pushing a swab through an untouched surface of the descending colon.

Swabs from the lung and spleen were plated on blood agar, chocolate agar, milk salt agar (Cruickshank, 1965) and MacConkey's agar; swabs from the colon were plated on MacConkey's agar only. Plates were incubated aerobically at 37°C. for 48 hr.; blood agar plates were duplicated for anaerobic culture. The approximate numbers of colonies on primary plates were counted and the organisms identified as fully as possible using the methods of Cowan & Steel (1965). Strains of *Staph. aureus* were phage typed and tested for sensitivity to penicillin and tetracycline and Gram-negative bacilli to sulphonamides, tetracycline, streptomycin and chloramphenicol, using lysed blood agar and 'Oxoid' sensitivity disks. The Oxford staphylococcus and a sensitive strain of *Escherichia coli* were included in each batch of tests as controls.

*Serological typing of* E. coli.

A very limited range of antisera was available; 01, 04, 06, 018*ab*, 018*ac*, 065, 069 and 075 were kindly supplied by Dr A. R. Foder of The Communicable Disease Centre, Atlanta, U.S.A.; 025 and 050 were prepared locally. The agglutination titres of these sera ranged from 1/2000 to 1/10,000.

For serotype determination, single colonies from primary cultures were subcultured on nutrient agar and, after biochemical confirmation of identity, saline suspensions were prepared, washed, boiled for 1 hr. and diluted to a final concentration of $250 \times 10^6$ organisms per ml. Equal quantities of suspension and antiserum diluted to one half titre were incubated at 50°C. in round-bottom tubes for 20 hr., when the presence of agglutination was read macroscopically. Five separate colonies of strains isolated from faeces were tested and two colonies of strains from other sites.

## RESULTS

In almost all of the 111 necropsies a serious underlying disease was present—mainly carcinoma or cardiovascular disease. Sixty-five patients had histological evidence of acute inflammation in the lungs, and of these 53 had pneumonia and 12 bronchitis, bronchiolitis or lung abscess. The remaining 46 patients had no histological evidence of pulmonary inflammation and were used as a control group.

Table 1 shows the relation between the presence of inflammatory lesions in the lung and the bacterial flora of the lung and spleen. It was expected that contaminant bacteria would occasionally be isolated from normal lung but that at least moderate numbers would be present in inflamed lung tissue; growths of less than 20 colonies per plate were therefore ignored. In fact it was found that most lung cultures were either sterile or exceeded 20 colonies per plate. The spleen on the other hand seemed less accessible to contamination and any bacterial growth was recorded.

Both *E. coli* and *Staph. aureus* were isolated significantly more frequently from inflamed lungs than from the lungs of controls ($P < 0{\cdot}0001$ and $P = 0{\cdot}0469$ respectively). *Haemophilus influenzae*, *Proteus mirabilis* and *Streptococcus pneumoniae* isolations had the same distribution but numbers were not significant. No anaerobic organisms were isolated.

Swabs from the spleen were cultured in the last 92 necropsies of the series, and of 53 patients with inflammatory lesions in the lung an organism was grown from the spleen in 25 whereas only eight of 39 controls were positive. Again, *E. coli* ($P = 0{\cdot}0267$) and *Staph. aureus* ($P = 0{\cdot}0050$) were significantly more frequent in patients

Table 1. *Bacteria isolated from the lungs and spleen from* 111 *necropsies*

| Bacteria isolated* | Lung. Pulmonary inflammation: Present, 65 patients | Lung. Pulmonary inflammation: Absent, 46 patients | Spleen. Pulmonary inflammation: Present, 53 patients | Spleen. Pulmonary inflammation: Absent, 39 patients |
|---|---|---|---|---|
| *Staph. aureus* | 20 (31) | 7 (15) | 12 (23) | 1 (3) |
| *E. coli* | 22 (34) | 2 (4) | 9 (17) | 1 (3) |
| *P. mirabilis* | 7 (11) | 3 (7) | 5 (9) | 1 (3) |
| Other coliform organisms† | 4 (6) | 3 (7) | 3 (6) | 1 (3) |
| *Str. pneumoniae* | 2 (3) | 0 | 0 | 1 (3) |
| β-haemolytic streptococci (groups B, C or G) | 2 (3) | 2 (4) | 1 (2) | 0 |
| *Str. faecalis* | 3 (5) | 2 (4) | 3 (6) | 0 |
| *Str. viridans* | 2 (3) | 5 (11) | 1 (2) | 2 (5) |
| *H. influenzae* | 5 (8) | 0 | 0 | 0 |
| *Past. haemolytica* var. *ureae* | 1 (2) | 0 | 0 | 0 |
| *Staph. saprophyticus* | 2 (3) | 2 (4) | 1 (2) | 3 (8) |
| No significant growth‡ or no growth | 20 (31) | 27 (59) | 28 (53) | 31 (80) |

The figures in parentheses are percentages in each group.
* A number of bacteria were present in mixed culture.
† Includes *Enterobacter aerogenes*. *Ent. cloacae*, *Klebsiella aerogenes* and Providence.
‡ See text.

Table 2. *Effect of time interval between death and necropsy on bacterial isolations*

| Pneumonic lesions | Site | Time between death and necropsy (hr.) | Number of necropsies | Number of patients yielding: *Staph. aureus* | Number of patients yielding: Entero-bacteria | Number of patients yielding: No significant growth |
|---|---|---|---|---|---|---|
| Absent | Lung | < 24 | 11 | 1 (9) | 2 (18) | 7 (64) |
| | | > 24* | 35 | 6 (17) | 5 (14) | 20 (57) |
| | Spleen | < 24 | 10 | 0 | 0 | 9 (90) |
| | | > 24† | 29 | 1 (3) | 3 (10) | 22 (80) |
| Present | Lung | < 24 | 12 | 3 (25) | 8 (67) | 4 (33) |
| | | > 24‡ | 53 | 17 (32) | 22 (42) | 16 (30) |
| | Spleen | < 24 | 11 | 2 (18) | 3 (27) | 5 (45) |
| | | > 24§ | 42 | 10 (24) | 13 (31) | 23 (55) |

Figures in parentheses indicate percentages in each group.
* Mean 2·1 days. Range 1–6 days.
† Mean 2·2 days. Range 1–6 days.
‡ Mean 1·7 days. Range 1–5 days.
§ Mean 1·8 days. Range 1–5 days.

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with pulmonary inflammation than in controls. Apart from the *Staph. saprophyticus* and *Str. viridans* other organisms showed the same distribution but differences were not significant.

Bacteria isolated from the spleen were usually also present in significant numbers in inflamed lungs. In fact 75 % of bacterial isolations from the spleen were accompanied by the isolation of an apparently identical species from the lung. Moreover, in 10 cases of *Staph. aureus* infection, the same phage type was present in both sites in eight cases; and in nine infections with typable *E. coli* the same serotype was isolated from both sites in eight.

The probabilities quoted above were calculated by Fisher's Exact Test (Fisher, 1950), using one tail only of the distribution. The isolation rates of *E. coli* from the lungs and of *Staph. aureus* from the spleen were significantly higher in the group with pulmonary inflammation by a one- or two-sided test. In view of the good correlation between isolations from the spleen and inflamed lung, the possibility of differences for these bacteria occurring in opposite directions in these two sites could be discounted. A single-sided test was therefore used for these particular probability estimates, but all subsequent tests were two-sided.

Table 3. *Effect of antibiotic therapy on pulmonary flora*

| Inflammatory lesions in lungs | Antibiotics given | Total no. of patients | No. of patients with bacteria in lungs | | | |
|---|---|---|---|---|---|---|
| | | | *E. coli* | *Staph aureus* | Other* organisms | All* organisms |
| Present | Yes | 34 | 7 (21) | 10 (29) | 11 (32) | 21 (62) |
| | No | 31 | 15 (48) | 10 (32) | 13 (42) | 24 (77) |
| Absent | Yes | 13 | 1 (8) | 2 (15) | 1 (8) | 4 (31) |
| | No | 33 | 1 (3) | 5 (15) | 3 (9) | 11 (13) |

Total necropsies, 111.
Numbers in parentheses indicate percentages of group total.
* *Str. viridans* and *Staph. saprophyticus* were considered to be of no significance and were excluded.

Necropsies were usually conducted within 2 days of death; 59 % were completed by the day after death and only 16 % were delayed for more than 3 days. There was no significant difference between isolation rates from necropsies conducted within 24 hr. of death and those in which there was delay (Table 2). The possibility that residual antibiotics in the tissues had prevented post mortem bacterial multiplication was considered, but it was found that similar proportions of all groups had received antibiotics.

Table 3 shows the effects of antibiotics on the pulmonary flora. *E. coli* was isolated more frequently from the patients with pulmonary inflammation who were not receiving antibiotics than those who were ($P = 0{\cdot}021$). It appeared therefore that antibiotics had probably suppressed the growth of *E. coli* in the treated group and that the isolation rate of 48 % in the untreated group was a better estimate of the incidence of *E. coli* infection.

The most commonly used antibiotic was tetracycline and the reduced *E. coli*

isolation rate in treated patients suggested that most strains were sensitive. Of the 15 strains of *E. coli* isolated from patients with pulmonary inflammation 12 were sensitive.

Antibiotic therapy made little difference to the isolation rates of *Staph. aureus* from the lungs. As none of the strains isolated from treated patients was sensitive to the antibiotic given, this was not surprising. Of 20 strains of *Staph. aureus* isolated from inflamed lungs 13 were tetracycline resistant and of these nine belonged to types which have often been associated with outbreaks of sepsis in surgical wards (eight 52/52A/80/81, 80/81 and similar patterns; one 84/85).

Using a very limited range of antisera, an attempt was made to determine the serotype of strains of *E. coli* isolated from inflammatory lesions of the lung and various other extra-intestinal sites in 25 patients (lung and spleen, eight patients; lung only, 13; spleen only, two; lung and rib abscess, one; liver abscess, one). Two colonies were tested from each site and in only two patients was a mixture of types demonstrated. Fourteen of 25 patients with *E. coli*-infected lesions had a typable strain in their lesion (06, 018, 050 or 075). The isolation rate from the faeces of these patients was similar; six of 12 tested carried one or other of these four strains in their faeces. On the other hand only one of 19 patients who did not have an *E. coli* infected lesion was carrying one of these strains (06, 018, 050 or 075) in their faeces.

An extrapulmonary lesion which could have been the source of Gram-negative metastatic bacterial infection was sought in the 53 patients with pneumonia. (Table 4.) Eight such lesions were found in 24 patients with enterobacterial pneumonia, whereas only one lesion ($P = 0{\cdot}0074$) was found in 29 patients with pneumonia associated with other bacteria.

Table 4. *Extrapulmonary lesions which could have been a source of enterobacteria*

(53 necropsies on patients with pneumonia)

| Lesion | Isolation of enterobacteria from lung: Present | Isolation of enterobacteria from lung: Absent |
|---|---|---|
| Perforated gastric carcinoma | 1 | — |
| Cholecystitis with liver abscesses | 1 | — |
| Diverticulitis of sigmoid colon | 1 | — |
| Pelvic abscess | 1 | — |
| Abscess associated with carcinoma of the colon | 1 | — |
| Necrotic strangulated bowel | 1 | — |
| Mesenteric thrombosis | 2 | — |
| Liver abscess | — | 1 |
| None | 16 | 28 |
| Total | 24 | 29 |

## DISCUSSION

For many years post mortem bacteriological investigations were thought to be of little value, as it was generally believed that the tissues were flooded with

bacteria, either before or after death. More recent work suggests that after the first few hours there is insignificant post mortem bacterial multiplication, and that whatever change in distribution of bacteria takes place at about the time of death its extent is not sufficient to obscure the true bacteriological picture (Burn, 1934; Smillie & Duerschner, 1947*a*; Kurtin, 1958; Kneeland & Price, 1960). Our results support this view; bacterial isolation rates from lungs and spleens changed insignificantly when necropsy was delayed and the correlation between histological and bacteriological results is incompatible with the possibility of the latter arising from random flooding with bacteria or from chance contamination due to the technique of swab collection.

It is difficult to assess the relative importance of different bacteria in the 65 patients with pulmonary inflammation because in 20 the bacterial growth was not significant, and in 21 it was mixed. However, *E. coli* was isolated most frequently and as antibiotics had reduced its isolation rate it would appear that nearly half the pneumonias were due to this organism.

*Staph. aureus* was isolated significantly more frequently from inflamed lungs than from the lungs of controls showing no inflammation. Although the controls had a high isolation rate, most were elderly patients with a serious underlying disease and 70% had histological evidence of pulmonary oedema which was perhaps premonitory of inflammatory change. A P.H.L.S. necropsy survey (Report, 1966) found that of 125 patients who died outside hospital only six (4·8%) had *Staph. aureus* in the lungs. It seems, therefore, that *Staph. aureus* might have been a contributory cause of death in something like 15% of patients with pneumonia.

Smillie & Duerschner (1947*b*) found that *Str. pyogenes*, *Str. pneumoniae*, and *H. influenzae* were the most common pathogens in terminal bronchopneumonia. *Staph. aureus* was frequently found, but it was also commonly present in the lungs of patients who did not have pneumonia; the authors concluded that it was sometimes a cause but less frequently than other organisms. These results were probably representative of the pre-antibiotic era, but subsequent reports indicated a changing pattern. Kneeland & Price (1960), and Lepper (1963), found that streptococci, pneumococci and *H. influenzae* had almost disappeared as causes of bronchopneumonia, their place being taken by *Staph. aureus* and to a lesser extent by Gram-negative bacilli. Our investigation also shows this pattern, and further suggests that *E. coli* may at times exceed *Staph. aureus* in frequency as a cause of terminal bronchopneumonia.

A question posed by this change in pattern is why has it occurred? Smillie & Duerschner (1947*a*, *b*) found that though organisms present in the nasopharynx could also be recovered from the trachea, they were not normally found beyond its bifurcation, but in terminal bronchopneumonia the more invasive of the nasopharyngeal bacteria passed this barrier to invade the lungs of patients whose resistance was impaired. Whether *Str. pyogenes*, *Str. pneumoniae* and *H. influenzae* have declined in virulence is uncertain, but perhaps a more important question is why they are being replaced by *Staph. aureus* and Gram-negative bacilli. Are they invading tissues from which they were previously excluded by more virulent bacteria? Or are the current hospital strains more virulent or prevalent than hitherto?

In the case of *Staph. aureus*, its increasing prevalence in terminal bronchopneumonia follows the recent trend of staphylococcal sepsis in general hospitals and is probably due to much the same factors. The most important of these is the high carriage rate among hospital patients and staff of virulent 'epidemic' types of staphyloccoci, the selection of which has been favoured by the use of antibiotics. Although our patients were in mental hospitals, the predominance of tetracycline-resistant strains and 'epidemic' phage types suggests that the staphylococcal environment was similar to that of general hospitals.

The large number of *E. coli* infections of the lung in our series was the most striking feature but the reason for it is not clear. A number of investigations have shown that only a few serological types of *E. coli* are responsible for most coliform infections (e.g. Turck, Petersdorf & Fournier, 1962; Kennedy, Plorde & Petersdorf, 1965). Much of this work has concentrated on urinary infection but our investigation suggests that in terminal bronchopneumonia there may be a high proportion of infections by certain serotypes (06, 075 and 018). Kennedy *et al.* (1965) and Winterbauer, Turck & Petersdorf (1967) found that types 04, 06 and 075 occur more frequently in the faeces of patients and staff in hospital and the carrier rate among patients was directly related to the time spent in hospital; inanimate objects in the wards were rarely contaminated and they therefore suggested that antibiotic treatment or other factors present in hospital patients might encourage spread from the endogenous bowel flora. Since, however, 40% of the hospital staff in their study were carrying these serotypes there would seem to have been ample opportunity for transmission by direct personal contact.

Whatever their source might be these serotypes readily colonise the faeces of hospital patients, but how do they reach the lungs? *E. coli* does not normally colonize the nasopharynx although it sometimes does so in infants. Recently, however, Stratford, Gallus, Matthiesson & Dixson (1968), reported that in severely ill patients the predominant bacterial flora of the skin, nose and throat changed from Gram-positive cocci to Gram-negative bacilli—mainly *E. coli* and *Proteus* spp. —and this change was not related to antibiotic therapy. If this occurs very commonly, invasion of the lungs via the air passages would be a likely route. Alternatively, bacteraemic spread from the gut or other extra-pulmonary inflammatory lesion could occur. Tillotson & Lerner (1967) reported 20 cases of pneumonia due to *E. coli* in which the bacilli were thought to reach the lungs via bacteraemias from sources in the kidneys or gastro-intestinal tract. In our series only eight of 22 patients with enterobacterial pneumonia were shown to have such a lesion, but it seems likely that this is an important route of infection (Table 4).

Whether the selection of these serotypes of *E. coli* is favoured by the use of antibiotics is uncertain. Since the strains isolated in this survey showed no increase in antibiotic resistance, we have no evidence of it, although Winterbauer *et al.* (1967) found that patients receiving broad-spectrum antibiotics had a tendency to acquire *E. coli* of O groups 4, 6 and 75 and felt that this was the result of alteration of the intestinal flora by antibiotics.

SUMMARY

One hundred and eleven consecutive deaths in seven mental hospitals during the months of February to October 1967 were investigated histologically and bacteriologically.

Bacteria were present in the lung tissue significantly more frequently when inflammation was present than when it was absent and the differences were significant for both *Escherichia coli* and *Staphylococcus aureus*; *E. coli* was isolated from 22 (34%) of the 65 patients with inflamed lungs compared with two (4%) of 46 control patients and the corresponding figures for *Staph. aureus* were 20 (31%) of 65 patients compared with seven (15%) of 46 control patients.

Some strains of *E. coli* were serotyped using antisera against O antigens 1, 4, 6, 18*ab*, 18*ac*, 25, 50, 65, 69 and 75 and the strains most commonly found in the inflamed lungs, spleens and other inflammatory lesions of 25 patients were 06, 018 and 075. These strains were isolated more frequently from these sites than from the faeces of a group of 19 patients with no such lesions.

In this series *E. coli* was the commonest organism to be associated with terminal bronchopneumonia and the possible reasons for this are discussed.

REFERENCES

BURN, C. G. (1934). Postmortem bacteriology. *J. infect. Dis.* **54**, 395.

COWAN, S. T. & STEEL, K. J. (1965). *Manual for the Identification of Medical Bacteria*. Cambridge University Press.

CRUICKSHANK, R. (1965). *Medical Microbiology*, 11th ed. Edinburgh and London: Livingstone.

FINLAND, M., JONES, W. F. & BARNES, M. W. (1959). Occurrence of serious bacterial infections since introduction of antibacterial agents. *J. Am. med. Ass.* **170**, 2188.

FISHER, R. A. (1950). *Statistical Methods for Research Workers*. Edinburgh: Oliver and Boyd.

KENNEDY, R. P., PLORDE, J. J. & PETERSDORF, R. G. (1965). Studies on the epidemiology of *Escherichia coli* infections. IV. Evidence for a nosocomial flora. *J. clin. Invest.* **44**, 193.

KNEELAND, Y. & PRICE, K. M. (1960). Antibiotics and terminal pneumonia. *Am. J. med.* **29**, 967.

KURTIN, J. J. (1958). Studies in autopsy bacteriology. *Am. J. clin. Path.* **30**, 239.

LEPPER, M. H. (1963). Opportunistic Gram negative rod pulmonary infections. *Dis. Chest* **44**, 18.

REPORT (1966). Necropsy survey of staphylococcal infection on patients dying in hospitals. A report from the Public Health Laboratory Service. *Br. med. J.* i, 313.

STRATFORD, B., GALLUS, A. S., MATTHIESSON, A. M. & DIXSON, S. (1968). Alteration of superficial bacterial flora in severely ill patients. *Lancet* i, 68.

SMILLIE, W. G. & DUERSCHNER, D. R. (1947*a*). The epidemiology of terminal bronchopneumonia. I. The significance of post-mortem cultures in determination of the etiology of terminal pneumonia. *Am. J. Hyg.* **45**, 1.

SMILLIE, W. G. & DUERSCHNER, D. R. (1947*b*). The epidemiology of terminal bronchopneumonia. II. The selectivity of nasopharyngeal bacteria in invasion of the lungs. *Am. J. Hyg.* **45**, 13.

TILLOTSON, J. R. & LERNER, A. M. (1967). Characteristics of pneumonias caused by *Escherichia coli*. *New. Engl. J. Med.* **277**, 115.

TURCK, M., PETERSDORF, R. G. & FOURNIER, M. R. (1962). The epidemiology of non-enteric *Escherichia coli* infections: prevalence of serological groups. *J. clin. Invest.* **41**, 1760.

WINTERBAUER, R. H., TURCK, M. & PETERSDORF, R. G. (1967). Studies on the epidemiology of *Escherichia coli* infections. V. Factors influencing acquisition of specific serologic groups. *J. clin. Invest.* **46**, 21.