J. Hyg., Camb. (1966), **64**, 65
Printed in Great Britain

Distribution of heat-resistant *Clostridium welchii* in a rural area of Australia

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(*Received* 16 *October* 1965)

The fact that *Clostridium welchii* occurs universally in nature has been well documented. Smith (1955) claims that *Cl. welchii* is more widely spread over the face of the earth than any other pathogenic bacterium. Taylor & Gordon (1940) reported finding *Cl. welchii* in 190 of 196 soil samples examined, the majority being type A. Hobbs (1962) found that the organism occurs regularly in dust—89·6 % of samples containing haemolytic and 81·0 % containing non-haemolytic *Cl. welchii*. Lowbury & Lilly (1958) found that *Cl. welchii* is regularly present in the hospital environment, being carried in from outside by dust and in the alimentary canal of patients.

The alimentary canal of humans and animals has long been known to contain *Cl. welchii*. Smith & Crabb (1961) found *Cl. welchii*, often in large numbers, in the alimentary canal of most farm and domestic animals, including dogs, cats, pigs and cows, and also in 80 % of humans examined. Collee, Knowlden & Hobbs (1961) and Taylor & Gordon (1940) both isolated *Cl. welchii*, using enrichment techniques in liquid medium, from 100 % of humans examined.

The above isolations have all been of the non-heat-resistant, haemolytic *Cl. welchii*, now considered part of the normal flora of man. The investigations by Hobbs *et al.* (1953), revealing heat-resistant non-haemolytic *Cl. welchii* as a cause of food poisoning, began a study by many to determine the carrier rate of this variant in the alimentary canal of man.

Figures for the general population include 2·2 % (Hobbs *et al.* 1953), 6–8 % (Collee *et al.* 1961), 14 % in male workers (Leeming, Pryce & Meynell, 1961) and 20 % in healthy hospital personnel and their families (Dische & Elek, 1957). Hospital patients appear to have unusually high carrier rates. Leeming *et al.* (1961) indicate that 30 % of hospital patients carry the organism, while Turner & Wong (1961) demonstrated heat-resistant spores in 63 % of Chinese hospital patients in Hong Kong—the same workers (using the same techniques) could demonstrate only a 9 % incidence of the organism in the general population of Leeds (England). Carrier rates are generally high following an infection—97 % at the time of infection (Dische & Elek, 1957) to 50 % 2 weeks after the infection (Hobbs *et al.* 1953). The variations in carrier rates expressed by different workers may be due to slightly different techniques, although the results of the examples cited above were all obtained by the use of very similar techniques. It does, however, seem apparent that the carrier rate is now higher in hospitals, boarding schools and other places of communal feeding than in the general population.

In the light of these results it was decided to determine the carrier rate of heat-resistant *Cl. welchii* in a rural area on the North Coast of New South Wales, Australia, and to gauge the effect of age, sex, season and hygiene on this carrier rate.

MATERIALS AND METHODS

The subjects

Faecal specimens were collected in sterile containers from five selected classes of the population.

Class A: primary-school children. Four hundred and sixty-one specimens were examined from non-aboriginal school-children, both male and female, ranging in age from 4 to 11 years. The children were from outlying country areas; the total enrolment at any one school being little more than thirty pupils. They therefore had little association with bulk-prepared canteen-type meals.

Class B: boarding-school students. Fifty-three specimens were examined from male students between the ages of 10 and 18 years. All were taken in mid-term, so that the students had been eating bulk-prepared food for several weeks. The above fifty-three students all attended the same school.

Class C: general public. This class consisted of fifty mildly ill persons attending their own general practitioner, and contained no aboriginal persons. Ages were not available in all cases, but they represent a good distribution of male and female persons from birth to 60 years of age.

Class D: hospital patients. This class consisted of forty-eight patients. A specimen of faeces was collected from each within 6 hr. of entering hospital (class Di) and a second specimen after they had been in hospital for 8 days (class Dii). Results of the two classes have been compiled separately as it was considered that although all specimens were from the same class of patients, they actually represent two distinct classes of persons living under different environmental conditions, particularly with regard to eating habits. The first specimen of faeces represents one from a member of the general public. The second specimen represents one from a member of a hospital environment.

Class E: aboriginals. Four hundred and twenty specimens were examined from aboriginals in four reserves and from a group living within the city, under conditions common to the white population. Two hundred and eighteen of the specimens were collected in the period November—April, and 202 were collected in the winter months of May, June and July.

Collection of material and culture methods

The majority of the faecal specimens were collected in sterile containers from the population classes A–E mentioned above. Samples from 150 primary-school children and 100 aboriginals were collected into clean, non-sterile containers. Numerous cultures on broth washings from such containers showed the absence of *Cl. welchii*, and the low incidence of heat-resistant *Cl. welchii* in primary-school children indicate that these containers introduced little or no *Cl. welchii*.

Where possible the stools were examined within 2 hr. of collection, but in a few cases, particularly those from an aboriginal reserve in an outlying area, they were not examined until 6–12 hr. after collection.

Media used. The egg-yolk medium was that of Willis & Hobbs (1958), with the medium base adjusted to pH 7·0. Other media used were blood agar (7·5 % defibrinated horse blood added to Oxoid blood-agar base) and Robertson's cooked-meat medium. Neomycin sulphate (Upjohn) was added to the egg-yolk medium and blood agar in a concentration of 100 μg./ml.

All faecal specimens received were examined for heat-resistant *Cl. welchii* by the method now commonly accepted by most bacteriologists (Hobbs *et al.* 1953, Leeming *et al.* 1961, Collee *et al.* 1961). One to two grams of all specimens were inoculated into a tube of cooked-meat medium which was then boiled for 60 min and incubated overnight at 37° C. In this way any heat-resistant spores present were concentrated. It was then subcultured on to egg-yolk medium and blood agar and incubated anaerobically for 24 hr.

On the egg-yolk medium colonies were 1–3 mm. in diameter, with a smooth texture and entire edge. They were cream on first isolation, surrounded by a zone of opalescence due to the lecithinase reaction, and a diffuse red zone due to the fermentation of the lactose in the medium. On standing the colonies themselves acquired a red colour. On blood agar the heat-resistant *Cl. welchii* showed little or no haemolysis. Colonies suspected of being *Cl. welchii* were further examined by gram stain, sugar fermentation tests and for the inhibition of the lecithinase reaction on egg-yolk medium by *Cl. welchii* antitoxin.

Serology

Antisera for Hobbs types 1–13 were kindly supplied by Dr H. Cherry of Communicable Disease Center, Atlanta, Georgia, U.S.A. Using these antisera the organisms classified as *Cl. welchii* were typed by the slide-agglutination technique; the sera were diluted within the range 1/5–1/15 (Hobbs *et al.* 1953).

RESULTS

Distribution of heat-resistant Cl. welchii *in various sections of the population of north eastern N.S.W.*

The results for classes A–E are shown in Table 1.

The figures given in Table 1 indicate that the six different classes of the population can be combined into two distinct larger groups.

Group 1: comprising classes A, C and Di. They all have a low carrier rate of heat-resistant *Cl. welchii* (1·5–6·0 %) and represent the majority of persons in the population.

Group 2: comprising classes B, Dii and E. They all have a high carrier rate (15·1–25 %) of heat-resistant *Cl. welchii*. This group consists of persons associated with either communal feeding or a low standard of hygiene and represents a minority group in the population.

Chi-squared tests performed on these two larger groups indicate that there is no significant difference between classes within the same group (Table 2).

Age distribution of positive specimens

This was only examined in the aboriginal population. In this class a large number of specimens was positive, and the figures therefore afford some comparison of the distribution of heat-resistant *Cl. welchii* among various age groups. The population has been divided into five age divisions (Table 3), each division

Table 1. *Heat-resistant* Clostridium welchii *infection rates of selected classes of the human population of north-eastern New South Wales*

Classes examined	No. specimens examined	Percentage positive
A	461	1·5
B	53	15·1
C	50	6·0
Di	48	4·2
Dii	48	25·0
E	420	19·0

A χ^2 test on these six classes gave a value of $\chi^2 = 79{\cdot}672$.

Table 2. *Some statistical data of results given in Table* 1

Group	χ^2	n	P
1	6·02	3	0·11
2	1·748	3	0·63

Table 3. *Distribution of heat-resistant* Clostridium welchii *among age groups of the aboriginal population*

Age group (years)	No. examined	Percentage positive
0–5	95	6·3
6–10	97	24·7
11–20	85	18·8
21–40	89	18·0
Over 40	54	33·0
Total	420	19·0

containing approximately the same number of persons. The numbers shown are a good representation of the aboriginal population, approximately 66 % being under the age of 21.

The results given in Table 3 were analysed statistically with the following results:

$$\chi^2 = 19{\cdot}424,\ n = 5,\ P = \text{less than } 0{\cdot}01,$$

which indicates that the difference in distribution of heat-resistant *Cl. welchii* among the various age groups is highly significant.

The age groups 0–5 ($t = 4{\cdot}09$, $P = < 0{\cdot}01$) and over 40 years ($t = 2{\cdot}09$, $P = 0{\cdot}94$) differ significantly from the overall aboriginal population.

Distribution of heat-resistant Cl. welchii *among male and female persons*

The results shown in Table 4 indicate that the distribution is fairly even; of 555 males examined 60 (10·8%) were positive, and of 525 females 52 (9·9%) were positive. Statistically the figures do not indicate any significant variation in carrier rates between males and females: $t = 0{\cdot}5$, D.F. $= \infty$, $P = 0{\cdot}62$.

Table 4. *Distribution of heat-resistant* Clostridium welchii *among males and females*

	Male		Female	
Group examined	No. examined	No. positive	No. examined	No. positive
A	221	4	240	3
B	53	8	—	—
C	27	2	23	1
Di	21	1	27	1
Dii	21	5	27	7
E	212	40	208	40
Total	555	60	525	52

Seasonal variation

Of the 218 aboriginal samples collected during the summer months 46 (21·0%) were positive, and 34 (16·8%) of the 202 examined in the winter months were positive.

Statistically the figures do not indicate a significant difference in the carriage of heat-resistant *Cl. welchii* during the summer and winter months ($t \doteqdot 1{\cdot}105$, D.F. $= 419$, $P = 0{\cdot}27$).

Serological types of Cl. welchii *isolated*

In order to study the epidemiology and carrier distribution of *Cl. welchii* with any degree of accuracy, all heat-resistant strains isolated, excepting twenty isolated from aboriginals early in the survey, were typed.

Some specimens were found to agglutinate with more than one serum, but in most of such cases one serum gave a stronger, more rapid agglutination than the others. Types 3, 4 and 5 demonstrated this phenomenon while types 9, 11 and 13 in many cases also cross-agglutinated.

No specimens fitted into serological types 4, 5, 7, 8 and 12, so these have been omitted from Table 5, which shows the complete typing results of the six classes. Unfortunately, because of conditions present at the time when this work was done, it was not possible to prepare rabbit antisera against the fifteen untypable specimens.

The carrier state and distribution within family groups

Twenty aboriginals from reserve A, positive in the original sampling, were resampled at 5 and 10 weeks to see whether the carriage of heat-resistant *Cl. welchii* is a transient or permanent state. The results indicate a transitory carriage. No patient carried the same strain in both resamples and in the original sampling.

https://doi.org/10.1017/S0022172400040341 Published online by Cambridge University Press

The figures in Table 6 show that after 5 and 10 weeks only 30 % of the specimens were positive. This is similar to the overall carrier rate of the reserve concerned, indicating that the twenty persons have now reverted to a random sample of the population, and no longer represent twenty selected (positive) persons. In addition, the table shows that almost all persons still positive at the resamples carried a different serotype from that isolated at the original sampling. This is evidence against a permanent carrier state.

Table 5. *Serological classification using Hobbs's typing sera* 1–13, *of heat-resistant* Clostridium welchii *isolated from selected classes of the population*

Hobbs's type	Class of patients A	B	C	Di	Dii	E
1	1	1	—	—	—	6
2	—	—	—	1	—	2
3	—	1	—	—	—	4
6	2	1	1	1	—	12
9	1	—	1	—	4	6
10	2	2	—	—	4	8
11	—	1	—	—	—	3
13	—	1	—	—	3	8
Untypable	1	1	1	—	1	11
Total	7	8	3	2	12	60

Table 6. *Result of specimens resampled* 5 *and* 10 *weeks after original sampling*

Time examined after original sample (weeks)	No. sampled	No. containing heat-resistant *Cl. welchii*	No. carrying same serotype as in original sampling
0	20	20	—
5	20	6	1
10	20	7	—*

* One specimen carried the same serotype as at 5 weeks but this differed from the serotype found in the original sampling.

One woman on entering hospital was a carrier of Hobbs type 6, but after 10 days in hospital this organism had been replaced by Hobbs type 9—present in three other members of her ward at that time. A further specimen, submitted 4 weeks after her hospital discharge, contained no heat-resistant *Cl. welchii*. In the hospital, where the two wards examined were served by the same kitchen, it was evident that one organism was predominantly present at any one time. Inspection of the serological types present in the hospital environment (Dii) shows that only three types were present. The presence of each type corresponded to a period of time, after which it was replaced entirely by a different serotype. The woman discussed above served as a fine example to illustrate this point.

No aboriginal reserve, as a whole, showed the carriage of any one predominant serotype. In cases where members of the same family were carriers of heat-resistant *Cl. welchii* there were many examples of the same serotype being carried by two or more members of the same family at the one time. In one case, however, three members of a family all carried different serotypes at the one time. There does, therefore, appear to be an association between carriage of heat-resistant *Cl. welchii* and certain families. Usually those families with poor hygienic conditions have several members carrying heat-resistant *Cl. welchii*. The seventy-eight families examined were divided into good, fair and poor hygiene, depending upon the general cleanliness of their dwellings, their persons and their habits. The use of refrigerators and other methods of food storage was also considered important. The distribution of heat-resistant *Cl. welchii* among these families is given in Table 7. A family in this case constitutes a group of persons living together and sharing the same meals.

Table 7. *Distribution of heat-resistant* Clostridium welchii *among aboriginal families with good, fair and poor hygiene*

Living conditions	No. of families examined	No. with one or more member infected	Percentage infected
Good hygiene	20	4	20
Fair hygiene	32	18	60
Poor hygiene	26	24	92

$\chi^2 = 26{\cdot}227$, $n = 3$, $P = < 0{\cdot}01$.

It is obvious from the results that a definite association between poor hygiene and the carriage of heat-resistant *Cl. welchii* among aboriginal families does occur.

Of the 26 families with poor hygiene, 24 had more than one member a carrier. There were 58 isolations of heat-resistant *Cl. welchii* from these 24 families. These results indicate that 72·5 % of positive specimens occur in 30·8 % of the families investigated. This is evidence for the hypothesis of carriage within family groups—those families concerned having a poor standard of hygiene. This, of course, does not mean that all carriers come from families with poor hygienic conditions. Most carriers within the white population acquire the organism from canteens, where conditions for correct food storage are not adequate, or not fully utilized. The aboriginals on the other hand have little association with canteens, and in most would acquire the organisms from their home environment.

DISCUSSION

The carrier rate recorded for the majority of persons on the north coast of N.S.W. (group 1, comprising classes A, C and Di) is lower than that recorded by many workers (Dische & Elek (1957), 20 %; Leeming *et al.* (1961), 14 %). This is probably due to the fact that the area is a predominantly rural one, with little industrial activity, and so with very little association with canteen-type food. It is noticeable however that persons associated with communal feeding (hospital patients, and boarding-school students) and with a low standard of hygiene (aboriginals) have a much higher carrier rate (15·1–25 %) than the majority of the

https://doi.org/10.1017/S0022172400040341 Published online by Cambridge University Press

population (1·5–6·0 %). These results are in agreement with those of Leeming *et al.* (1961) who demonstrated an increased carrier rate in hospital patients, and with Turner & Wong (1961) who demonstrated 63 % carriage of heat-resistant *Cl. welchii* in Chinese hospital patients of Hong Kong and only 9 % in the general population of Leeds (England). Selection and transmission of heat-resistant strains by bulk cooking followed by slow cooling of the meat could partly account for this predominance of heat-resisant *Cl. welchii* in hospitals and institutions. In the aboriginal persons examined it was evident that age was a significant factor in the distribution of heat-resistant *Cl. welchii* ($\chi^2 = 19{\cdot}424$). The age groups 0–5 and those over 40 years differ significantly from the mean aboriginal carrier rate ($t = 4{\cdot}09$ and $2{\cdot}09$ respectively). This difference in the carriage of heat-resistant *Cl. welchii* is probably related to differences in eating habits of the various age groups. This applies particularly to the 0–5 age groups, where most are breast-fed in infancy, and even up to the age of 3 or 4 years eat specially prepared meals, and do not partake in the normal family meal. Results show that the distribution of the organism among males and females is similar. Leeming *et al.* (1961) were able to demonstrate a higher carrier rate in men, but explained that this was probably due to the eating habits—the men tending to eat a large number of canteen-prepared meals. In hospital patients the male and female carrier rates became the same. There would therefore appear to be no reason (other than eating habits) why male and female persons should not have similar carrier rates of heat-resistant *Cl. welchii.* All this work clearly indicates that the carriage of heat-resistant *Cl. welchii* is closely linked with communal feeding and poor hygienic conditions ($\chi^2 = 26{\cdot}227$ for hygiene); in both cases the food eaten is often left unrefrigerated for some hours between cooking and the actual consumption. The Ministry of Health (Report, 1964) reports that 60 of 62 outbreaks of food poisoning due to *Cl. welchii* occurred in either hospitals, school canteens, restaurants or institutions.

Particularly interesting was the result indicating that there was no significant difference in the carriage of heat-resistant *Cl. welchii* in summer or winter months. This is not in agreement with the occurrence of food-poisoning due to other organisms. Wilson & Miles (1955) point out that cases of bacterial food-poisoning are most frequent in the summer months. In England and Wales during 1963 (Report, 1964) 47 % of *Salmonella* food-poisoning outbreaks occurred during the months June to August, and all staphylococcal outbreaks occurred from March to October. On the other hand, the same table of results indicates that outbreaks of *Cl. welchii* food-poisoning occurred regularly throughout the year.

This may be due to the fact that in most cases of *Cl. welchii* food-poisoning the meat dish is naturally contaminated before it reaches the kitchen. The food handler plays little part in the contamination of the food. The heat-resistant spores are able to withstand the cooking process, particularly if the portion of meat being cooked is large and bulky. As the meat dish cools, the temperature of the meat drops to 50° C. within 2½ hr. but then cools more slowly, remaining within the temperature range of 20–50° C. for many hours (McKillop, 1959). This is particularly so in a warm kitchen, where several roasts are allowed to stand together after cooking. This offers ideal opportunities for the heat-resistant spores, already present, to germinate and multiply. Collee *et al.* (1961) were able to demonstrate that heat-resistant *Cl. welchii* grows rapidly within a temperature range of 23–50° C. In the cold winter months, if the meat be drained of its broth and put aside from other portions of meat, thus allowing it to cool more rapidly, it is probable that the infection would be less prevalent. The ideal solution, of course, both in summer and winter months, is to drain the meat of its broth, and refrigerate it immediately after cooking.

Most attention has been focused on the organism as a cause of general outbreaks. The British Ministry of Health (Report, 1964) lists *Cl. welchii* as causing 42 % of all general, but only 3 % of family outbreaks of food-poisoning occurring in England and Wales during 1963. This is probably due to the actual method of contamination and subsequent multiplication; conditions being favourable in institutional environments, in which most general outbreaks occur. Results given earlier in this paper indicate that carriage within a family group is common—72·5 % of positive specimens from aboriginals occurring in 30·8 % of the families investigated. Families with poor hygiene often have two or three members carrying heat-resistant *Cl. welchii* at the one time, while those with good hygiene have little association with the organism. Turner & Wong (1961) found a 63 % carrier rate in persons who, although in hospital, otherwise have very little association with canteen-type foods, and generally live in large family groups under poor hygienic conditions. Such results suggest that *Cl. welchii* must be considered as a source of food-poisoning within the family, particularly families living under poor hygienic conditions. As the infection is mild, and lasts only 24 hr. the average person would not contact his doctor and such a family outbreak would go unnoticed. In hospitals and boarding schools, however, diarrhoea in a large number of persons at the same time can hardly go unnoticed.

SUMMARY

The incidence of heat-resistant *Cl. welchii* in selected classes of a rural population has been investigated. The carrier rate for the general population was low (1·5–6·0 %), but persons associated with communal feeding and poor hygienic conditions were shown to have a much higher carrier rate (15·1–25 %).

Sex and season had little effect on the incidence of the organism, but among aboriginals the carriage was significantly affected by age. This was probably due to eating habits of the different age groups. The carriage appears to be transient; no persons were shown to be permanent carriers of one strain of heat-resistant *Cl. welchii*.

I wish to acknowledge the helpful advice given by Drs B. McMillan, M. A. Bundock, Phyllis Rountree and Betty C. Hobbs, and Dr Cherry of the Communicable Disease Center, Atlanta, Georgia, U.S.A., for the supply of typing sera. I would also like to express my thanks to the Commonwealth Director General of Health for permission to publish this work.

REFERENCES

COLLEE, J. G., KNOWLDEN, J. A. & HOBBS, B. C. (1961). Studies of growth, sporulation and carriage of *Cl. welchii* with reference to food poisoning strains. *J. appl. Bact.* **24**, 326.

DISCHE, R. E. & ELEK, S. T. (1957). Experimental food poisoning by *Clostridium welchii. Lancet*, ii, 71.

HOBBS, B. C. (1962). Staphylococcal and *Clostridiun welchii* food poisoning. In *Food Poisoning: Symposium*, p. 49. London: Royal Society of Health.

HOBBS, B. C., SMITH, M. E., OAKLEY, C. L., WARRACK, G. H. & CRUICKSHANK, J. C. (1953). *Clostridium welchii* food poisoning. *J. Hyg., Camb.*, **51**, 75.

LEEMING, R. L., PRYCE, J. D., MEYNELL, M. J. (1961). *Clostridium welchii* and food poisoning. *Br. med. J.* i, 50.

LOWBURY, E. J. L. & LILLY, W. A. (1958). Source of hospital infection of wounds with *Cl. welchii. J. Hyg., Camb.*, **56**, 169.

MCKILLOP, E. J. (1959). Bacterial contamination of hospital food, with special reference to *Cl. welchii* food poisoning. *J. Hyg., Camb.*, **57**, 31.

REPORT (1964). Food poisoning in England and Wales, 1963. *Mon. Bull. Minist. Hlth*, **23**, 189.

SMITH, H. W. & CRABB, W. E. (1961). The faecal bacterial flora of animals and man: its development in the young. *J. Path. Bact.* **82**, 53.

SMITH, L. DE S. (1955). *Introduction to the Pathogenic Anaerobes*. University of Chicago press.

TAYLOR, A. W. & GORDON, W. S. (1940). A survey of the types of *Cl. welchii* present in soil and in the intestinal contents of animals and man. *J. Path. Bact.* **50**, 271.

TURNER, G. C. & WONG, MING, M. (1961). Intestinal excretion of heat-resistant *Clostridium welchii* in Hong Kong. *J. Path. Bact.* **82**, 529.

WILLIS, A. T. & HOBBS, G. (1958). A medium for the identification of Clostridia producing opalescence in egg yolk emulsions. *J. Path. Bact.* **75**, 299.

WILSON, G. S. & MILES, A. A. (1955). *Topley and Wilson's Principles of Bacteriology and Immunity*, 4th ed., pp. 1795–6. London: Edward Arnold Ltd.