J. Hyg., Camb. (1978), **81**, 161
Printed in Great Britain

A study of bacteria contaminating refrigerated cooked chicken; their spoilage potential and possible origin

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(*Received* 12 *August* 1977)

SUMMARY

Cooked chicken was allowed to spoil in a normal kitchen refrigerator (variable temperature) and at a standard 4 °C. After 10 days' storage, bacteria were isolated from the chicken. It was found that the numbers of organisms at variable refrigeration temperature were tenfold higher than those at a uniform 4 °C. In an attempt to find the sources of contamination, swabs were made of different areas of the kitchen. Many of the bacteria isolated from the spoiled chicken, were also isolated from the kitchen environment.

When pure cultures of organisms isolated from spoiled chicken were inoculated into sterile cooked chicken and held at 4 °C, the main spoilage organisms were found to be *Pseudomonas putida* and *Aeromonas hydrophila*, which were also isolated from the refrigerator where the chickens were stored in the kitchen.

Aeromonas hydrophila was found in significantly high numbers on plates, cutting knives, chopping boards and cold water taps.

INTRODUCTION

Micro-organisms associated with spoilage of uncooked poultry are psychrophilic organisms – predominantly *Pseudomonas* sp (Nagel *et al.* 1960). Patterson & Gibbs, (1973) showed that after cooking most non-sporing organisms were destroyed, organisms surviving cooking being *Bacillus* sp; *Staphylococcus epidermidis* was also isolated.

The shelf-life of raw chicken depends on the actual storage temperature, types of psychrophils present, and their growth rate at the temperature used. Walker & Ayres (1956) showed that initially *Pseudomonas* formed only 40 % of the population on raw poultry, but after 2 weeks' storage at 4 °C had selectively increased to form 95 % of the total population. Many more organisms grow at 4 °C than below 0 °C, suggesting the need for storage of poultry below this temperature. Barnes (1976) showed the differences in time for development of 'off-odours' and hence spoilage of raw chicken at different temperatures, i.e. 'off-odours' developed in 7·2 days at 5 °C, and 38 days at −2 °C.

The shelf-life of chicken is affected by pH and type of muscle, type of packaging, and presence of CO_2 as well as by storage temperatures. Barnes (1976) showed that extending the shelf-life of chickens depends on delaying or inhibiting pseudomonads – by type of packaging, 10–20 % CO_2 or irradiation or antibacterial compounds, e.g. chlortetracycline.

https://doi.org/10.1017/S0022172400024980 Published online by Cambridge University Press

While initial flavour quality is important, the keeping quality of chicken must be adequately maintained during storage, distribution and retail handling. Cash & Carlin (1968) found that rancidity was significantly affected by pre-cooking. Values for fresh frozen controls were always lower than for pre-cooked stored samples.

Borgstom (1955) studied the problem of cooked foods compared with raw foods. Cooked foods are very easily contaminated and utilized by micro-organisms because the structure of the tissue is soft when compared with raw products. Cooking will destroy some bacteria, but there are many opportunities for contamination during subsequent handling. May (1962) studied the bacterial contamination that occurred in chicken during cutting and packaging in commercial plants and retail stores.

The cooked product presents a different range of problems from those of the raw product. Although cooking will destroy most micro-organisms, the product is easily contaminated after cooking, both by the handler, the utensils and surfaces in the kitchen environment. Although there is a danger of food poisoning from such contamination, this is not as likely as food spoilage. The storage temperature will affect the shelf-life of the cooked chicken. To prevent growth of food poisoning organisms, the product should be stored under refrigerated conditions. However, these provide ideal conditions for the growth of psychrophilic spoilage organisms, so that cooked chicken may have a very short shelf-life, often no more than 2 days at 4 °C. After this time, the increase in numbers of spoilage bacteria is rapid, producing visible changes in appearance of the chicken, and disagreeable odours and taste, making the product unacceptable.

The contamination of chicken after cooking is therefore a great problem, and if it could be stopped or even lessened, then the storage life could presumably be increased. Therefore sources of contamination occurring in the kitchen environment were studied.

MATERIALS AND METHODS

Cooked chicken samples were supplied by the Hotel and Catering Department, University of Surrey. Samples were divided into three portions; the first was covered with commercial 'cling-film' wrap and stored in the Hotel and Catering refrigerator (the temperature being measured continuously by a thermograph), the second was treated similarly and stored at constant 4 °C. Both were stored for 10 days after which time they were removed for bacteriological examination. The third portion was removed for bacteriological examination immediately after cooking.

For bacteriological examination the portions were placed in previously weighed sterile Universal bottles and the weights of the samples were determined. The samples were then homogenized in twice their weight of 0·1 % peptone water solution (Avens & Miller, 1970) for 2 min in a 'Stomacher'. A series of tenfold dilutions was made, down to 10^{-5}.

The chicken sample examined immediately after cooking was cultured on plate count agar (using a 0·1 ml inoculum spread over the plate) incubated at 20 °C for

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Table 1. *Bacteria isolated from cooked chicken – sampled immediately after cooking*

(The weight of chicken sampled was 18·1 g.)

Media and incubation temp. and time	Genera	Colonies (g)	Total count (%)
Plate count Agar 20 °C (7 days)	Yeast? Rhodotorula	$1{\cdot}5\times10^{2}$	2·7
	Pseudomonas cepacia	$1{\cdot}5\times10^{2}$	2·7
	Micrococcus varians	$4{\cdot}2\times10^{3}$	75·0
	Lactobacillus sp.	$1{\cdot}1\times10^{3}$	19·6
	Total count	$5{\cdot}6\times10^{3}$	—
Plate count Agar 4 °C (10 days)	*Pseudomonas cepacia*	$1{\cdot}5\times10^{1}$	—

7 days and at 4 °C for 10 days. For chicken samples stored for 10 days a wider range of selective media was used as more different species of bacteria were expected. These were McConkey agar, nutrient agar + 7·5 % NaCl and mannitol salt agar, all incubated at 37 °C for 2 days, trypticase soy agar and plate count agar at 20 °C for 5 days, and plate count agar at 4 °C for 10 days. In each case 0·1 ml was spread over the plate.

The bacterial flora of the kitchen environment was examined by means of alginate wool swabs (Higgins, 1950). Areas examined were working surface, chopping board, knife, sink, refrigerator, floor tile, plate, taps in sink and knife sharpener. Also examined were the air of the kitchen and refrigerator, and a hand impression plate of one of the kitchen staff.

Inoculation experiments using sterile chicken

Pure cultures of organisms isolated from the kitchen were inoculated into sterile chicken by completely immersing the chicken sample in the appropriate dilution of the culture. A low (10^{-1}) and high (10^{-5}) dilution of each bacterial culture was used for each chicken piece. After storage at 4 °C the inoculated chicken pieces were examined for appearance and odour. Spoilage was defined as the time for definite 'off-odours' to occur, judged by an assessment panel.

The highest temperature observed in the refrigerator was 13 °C and the lowest 2 °C. Every morning the temperature rose to at least 10 °C and this was maintained for 5 to 7 h. This rise was due partly to the frequent opening of the door during cooking hours when the kitchen was exceptionally hot and humid, and partly to the fact that many products were put in the refrigerator whilst still hot to aid rapid cooling.

Flora of the chicken samples

Chicken examined immediately after cooking

The organisms are shown in Table 1 where it is seen that the counts are low, about 10^{2}–10^{3} organisms/g. This agrees with findings by Barnes (1976) studying uncooked chicken.

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Table 2. *Bacteria isolated from cooked chicken – sampled after storage in Hotel and Catering refrigerator (variable temperature) for* 10 *days*

(The weight of chicken sampled was 26·5 g.)

Media and incubation temp. and time	Genera	Colonies (g)
McConkey agar 37 °C (2 days)	*Staph. epidermidis*	$4·0 \times 10^2$
	Bacillus sp.	$6·0 \times 10^3$
	Micrococcus varians	$1·2 \times 10^3$
	Micrococcus roseus	$4·0 \times 10^4$
Nutrient agar + 7·5 % NaCl 37 °C (2 days)	*Streptococcus* sp.	$3·0 \times 10^4$
Mannitol salt agar 37 °C (2 days)	*Aerococcus* sp.	$2·4 \times 10^4$
	Staph. epidermidis	$4·0 \times 10^2$
Trypticase soy agar 20 °C (5 days)	*Pseudomonas fluorescens*	$6·7 \times 10^7$
Plate count agar 20 °C (5 days)	*Pseudomonas putida*	$2·1 \times 10^7$
Plate count agar 4 °C (10 days)	*Aeromonas hydrophila*	$2·8 \times 10^7$
	Pseudomonas putida	$1·1 \times 10^7$

Table 3. *Bacteria isolated from cooked chicken – sampled after storage in 'cold room'* (4 °C) *for* 10 *days*

(The weight of chicken sampled was 27·6 g.)

Media and incubation temp. and time	Genera	(Colonies g)
McConkey agar 37 °C (2 days)	*Staph. epidermidis*	$7·6 \times 10^2$
Nutrient agar + 7·5 % NaCl 37 °C (2 days)	*Streptococcus* sp.	$2·0 \times 10^3$
Mannitol salt agar 37 °C (2 days)	*Staph. epidermis*	$2·0 \times 10^1$
	Micrococcus roseus	$6·6 \times 10^2$
Trypticase soy agar 20 °C (5 days)	*Pseudomonas putida*	$4·2 \times 10^6$
Plate count agar 20 °C (5 days)	*Pseudomonas putida*	$3·8 \times 10^6$
	Corynebacterium sp.	$4·6 \times 10^5$
Plate count agar 4 °C (10 days)	*Aeromonas hydrophila*	$6·0 \times 10^6$
	Pseudomonas putida	$4·6 \times 10^6$

Chicken examined after 10 *days' incubation in the Hotel and Catering refrigerator (variable temperature)*

The flora is shown in Table 2. Only *Pseudomonas putida*, *Ps. fluorescens* and *Aeromonas hydrophila* were present in large numbers, the other organisms being present in very low numbers. This contrasts with the sample examined immediately after cooking, where *Micrococcus varians* was present in relatively high numbers, and *Pseudomonas* in very low numbers.

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Table 4. *Counts of bacteria obtained from swabbing the kitchen environment*

	Colonies/cm^2	
Area of Swabbing	Trypticase soy agar 30 °C (2 days)	Plate count agar 20 °C (3 days)
Working surface	$2\cdot2 \times 10^2$	$3\cdot8 \times 10^2$
Chopping board	$1\cdot2 \times 10^4$	$6\cdot7 \times 10^3$
Knife	$2\cdot8 \times 10^3$	$5\cdot8 \times 10^2$
Sink	$1\cdot4 \times 10^3$	$2\cdot0 \times 10^2$
Refrigerator	$5\cdot6 \times 10^2$	$3\cdot6 \times 10^2$
Floor	$1\cdot2 \times 10^3$	$1\cdot1 \times 10^2$
Plate	$3\cdot2 \times 10^2$	$8\cdot0 \times 10^1$
Taps in sink	$4\cdot6 \times 10^4$	$6\cdot3 \times 10^3$
Knife sharpener	$6\cdot0 \times 10^2$	$5\cdot0 \times 10^2$

Chicken examined after 10 *days' incubation at* 4 °C.

The flora is shown in Table 3. *Pseudomonas putida, Aeromonas hydrophila* and *Corynebacterium* sp. were present in large numbers, the other species in significantly lower numbers. The lower storage temperature of the chicken served to restrict the total numbers of bacteria tenfold, and also restricted the number of genera isolated, i.e. *Bacillus* sp., *Micrococcus varians, Aeromonas* sp. and *Pseudomonas fluorescens* were not isolated from the sample stored at 4 °C, but were isolated from that stored at variable temperature.

Although the work described was concerned with cooked chicken, the organisms found in high numbers were *Pseudomonas* and *Aeromonas* sp., as described by Barnes (1976) working with raw chicken, rather than the heat resistant organisms described by Patterson & Gibbs (1973). Sporing bacteria e.g., *Bacillus* sp. were isolated, but only from the chicken sample stored at the higher variable temperature, and formed 0·1 % of the total flora.

The fact that *Pseudomonas* could be isolated after cooking, even though present in very low numbers, suggests that there might have been insufficient cooking of the chicken. However, although *Pseudomonas* was isolated after cooking, it was not the same species that was isolated after spoilage. As the number of heat-resistant bacteria isolated from spoiled chickens was not significant (0·1 %) it would suggest that bacteria causing spoilage of the chicken had arisen as a result of contamination after cooking.

Flora isolated from the kitchen environment

The total number of bacteria isolated from swabs is summarized in Table 4.

Bacteria isolated from individual swabs are shown in Table 5, as the percentage found on each swab. The total flora isolated were: *Pseudomonas fluorescens, Flavobacterium* sp. *Bacillus* sp., *Aeromonas hydrophila, Proteus* sp., *Pseudomonas putida, Aeromonas, Arthrobacter* sp. and *Microbacterium thermosphactum.*

From the air in the kitchen *Pseudomonas fluorescens, Flavobacterium* sp. and *Bacillus* sp. were isolated. The air in the refrigerator contained *Pseudomonas fluorescens* and *Aeromonas hydrophila.*

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Table 5. *Relative percentages of organisms obtained on each swabbing area of the kitchen*

Swabbing area	1	2	3	4	5	6	7	8	9	10	11
Working surface	—	—	—	33·3	33·3	—	—	—	—	—	33·3
Chopping board	—	—	—	—	—	—	—	100	—	—	—
Knife	—	—	—	80·9	4·8	3·2	—	—	—	—	11·1
Sink	—	—	50	—	50	—	—	—	—	—	—
Fridge	60·7	3·6	10·7	—	—	—	25	—	—	—	—
Floor	—	—	91·7	—	8·3	—	—	—	—	—	—
Plate	—	—	—	66·6	16·7	—	—	—	—	—	16·7
Taps	—	—	—	3·4	0·8	—	93·1	—	—	—	2·7
Knife sharpener	—	—	—	37·5	37·5	—	—	—	—	—	25
Air in kitchen	31·0	4·4	2·2	—	—	—	—	—	—	—	62·4
Air in fridge	14·3	—	—	14·3	—	—	—	—	—	—	71·4
Hand impression	—	—	—	—	—	—	—	—	70·7	29·3	—

Colony types: 1, *Pseudomonas fluorescens*; 2, *Flavobacterium* sp.; 3, *Bacillus* sp.; 4, *Aeromonas hydrophila*; 5, *Proteus* sp.; 6, *Pseudomonas putida*; 7, *Arthrobacter* sp.; 8, *Microbacterium thermosphactum*; 9, *Staph. epidemidis*; 10, *Corynebacterium* sp.; 11, Fungal colonies.

A hand impression plate of the carver showed *Staph epidermidis* and *Corynebacterium* sp.

It was found that spoilage had not occurred as a result of bad handling, as *Staph epidermidis* and *Corynebacterium* sp. were only isolated from hand impression plates, and formed less than 0·1 % of the total flora of the spoiled chicken.

However, it is significant that *Aeromonas hydrophila* formed up to 56 % of the total flora isolated from the spoiled chicken, and was present in a range of areas of the kitchen. It was isolated from working surfaces, cutting equipment, on 'clean' plates, and in the air in the refrigerator where the food was stored. It would therefore be very easy for this organism to contaminate the cooked chicken at many stages of handling and storage.

It was also significant that *Pseudomonas fluorescens* and *Aeromonas hydrophila* were found to be able to grow and multiply rapidly in foods stored at 4 °C; these organisms were isolated from the refrigerator, which itself could serve as a source of contamination for other foods.

Ability of pure cultures to cause spoilage of cooked chicken

Pure cultures of bacteria previously isolated from cooked stored chicken were used to inoculate autoclaved chicken. The results are summarized in Table 6. The original inoculum of bacteria used for the sterile chicken was 10^{-1}, 10^{-2} or 10^{-3} for the high inocula, and 10^{-5}, 10^{-6} or 10^{-7} for the low inocula. Spoilage was determined by smell of the chicken, compared with that of an uninoculated piece of autoclaved chicken.

Cultures producing spoilage of the chicken

'Off-odours' were produced from 5–18 days, depending on the original inoculum of bacteria, and the type of organism. The organisms producing detectable spoilage in this time were: *Aeromonas hydrophila*, *Pseudomonas putida*, *Arthro-*

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Table 6. *Inoculation of sterile chicken with pure cultures of the bacteria isolated from the kitchen environment*

Genera	Original number inoculated	Number at time of 'off-odours' per gram/wt	Time taken for 'off-odour'	Type of odour
Pseudomonas fluorescens	1.3×10^7	2.9×10^8	12 days	'Rotting
	1.3×10^3	5.3×10^7	15 days	veg'
Flavobacterium sp.	8.1×10^6	1.2×10^9	12 days	Fishy
	8.1×10^2	3.8×10^8	15 days	
Bacillus sp.	1.4×10^5	No 'off-odours' – contamination		
	1.4×10^1			
Aeromonas hydrophila	2.5×10^6	1.1×10^{10}	5 days	Putrid
	2.5×10^2	8.5×10^9	9 days	ammonia
Proteus sp.	7.0×10^6	No 'off-odours' – contamination		
	7.0×10^2			
Pseudomonas putida	5.6×10^6	1.1×10^9	5 days	Putrid
	5.6×10^2	4.6×10^9	9 days	ammonia
Arthrobacter sp.	9.1×10^6	5.6×10^7	9 days	'Off'
	9.1×10^2	2.8×10^7	18 days	
Microbacterium thermosphactum	1.0×10^6	1.1×10^7	18 days	
	1.0×10^2	No 'off-odours'		
Staph epidermidis	4.1×10^6	No 'off-odours' – contamination		
	4.1×10^2			
Corynebacterium sp.	6.1×10^6	5.0×10^7	18 days	'Off'
	6.1×10^2	2.0×10^3	No 'off-odour'	
Control	No inoculum	None	No 'off-odour'	

bacter sp., *Pseudomonas fluorescens*, *Flavobacterium* sp. and *Microbacterium thermosphactum*.

Corynebacterium sp., produced slight 'off-odour' in the chicken inoculated with a high number of organisms, but not in chicken inoculated with low numbers.

Cultures producing no spoilage of the chicken

Bacteria causing no visible spoilage or 'off-odours' of the chicken after incubation at 4 °C for 21 days were: *Bacillus* sp. *Proteus* sp., *Staph epidermidis*, and low inocula of *Microbacterium thermosphactum* and *Corynebacterium*.

The cultures causing no spoilage were not present in pure cultures when re-isolated from the chicken, but the chicken inoculated with those organisms were found to be contaminated by yeasts, gram +ve cocci and gram +ve rods – probably present in the air in the laboratory, and in the refrigerator where the chicken was kept.

The autoclaved chicken was shown to be sterile before inoculation, as a control piece of chicken (no inoculum), gave no appearance of spoilage at the end of the incubation time, and no bacteria were isolated from it when cultured.

The length of time for 'off-odours' to occur depended on the size of the original inoculum. Ingram & Dainty (1971) showed that no clear changes in composition of

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meat occurred until 10^8 bacteria/cm^2 were present. In the present study, for an original inoculum of approximately 10^2 bacteria, an extra 4 days were needed before spoilage occurred (compared with an inoculum of approximately 10^6 bacteria), due to the time needed for the initial increase in numbers of bacteria.

Thus, spoilage could be attributed to psychrophilic bacteria, able to multiply rapidly at 4 °C. The most important spoilage organisms were *Pseudomonas putida* and *Aeromonas hydrophila*, which caused spoilage much more rapidly when present in pure cultures in cooked chicken.

Most of the spoilage organisms are normal inhabitants of soil and water, therefore can easily enter the kitchen, perhaps on vegetables, or on soil brought in on footwear, or in the cold water supply.

Suggestions for improving hygiene and keeping quality of cooked meats and poultry

The variations in temperature of the refrigerator have important implications in kitchen management. The variable temperature range (2–13 °C) resulted in a greater number of bacterial species on the cooked chicken and also in total counts of bacteria which were tenfold higher than in chicken stored at a standard 4 °C. The resulting higher numbers of bacteria would cause spoilage in a shorter time, often 4 or 5 days sooner (see Table 6). Thus, if a standard 4 °C could be maintained in the storage refrigerator, numbers of bacteria would be lower and the keeping quality of cooked chicken would be improved. This could be achieved by keeping the refrigerator away from the humid atmosphere of the ovens, preferably in a different room and restricting occasions of door opening.

Patterson & Gibbs (1973), working on cooked chicken, did not demonstrate the presence of pseudomonads after spoilage, possibly due to their aseptic handling of the chicken after cooking. However, in this study of cooked chicken, kitchen staff were requested to cut up and store the chicken using their normal methods and equipment. Although the environment and equipment were visually clean and attractive and the staff were competent and knowledgeable, the work demonstrated that cleanliness was not sufficient to prevent spoilage organisms contaminating cooked food. In particular, knives, storage plates and washing facilities were heavily contaminated with *Aeromonas* sp., able to cause spoilage of cooked chicken within 5 days at 4 °C.

The highest count of bacteria was obtained from the taps in the sink, from which *Aeromonas hydrophila* were isolated, hence it is feasible that utensils might be washed and thus inoculated with organisms capable of causing spoilage – then used for cutting or storing cooked foods. This would occur especially where cold or luke-warm water was used in rinsing. A positive recommendation to wash kitchen utensils in hot (80 °C) water, and allow to dry before use, might reduce initial contamination.

The results showed that although a range of organisms was isolated from spoiled chicken, only a few species were actually responsible for spoilage; *Aeromonas hydrophila* and *Pseudomonas putida* being most important in this respect. These organisms were found to be widely distributed in the kitchen environment –

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especially in the refrigerator. If *Aeromonas* and *Pseudomonas* could be eliminated from the environment, rapid spoilage of cooked chicken could be prevented, and the shelf-life of the chicken might be extended. Other organisms were found to cause spoilage, but needed a significantly longer time. Therefore, a doubling or trebling of safe refrigeration time from 2–6 days would be possible, although carefully regulating the storage temperature at 4 °C or below, as well as reducing the initial contamination, would be necessary.

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