Comparative Microflora of Chlortetracycline-treated and Nontreated Poultry with Special Reference to Public Health Aspects

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Received for publication April 18, 1960

This paper reports the outcome of experiments to determine whether, under commerical conditions as applied in Canada, the use of chlortetracycline (CTC) as a preservative of poultry meat could be shown to have increased the frequency of occurrence within the poultry of salmonellae, staphylococci, or of pathogenic yeasts. Concomitantly, a study was also made of the comparative predominance among CTC-treated and nontreated poultry of specific groups of saprophytic microorganisms, including the fecal "indicators," *Escherichia coli* and enterococci. The resistance to CTC was determined for all pathogenic isolates and for representatives of each group of indicator and spoilage organisms. The more resistant of this last category were classified as to genus.

MATERIALS AND METHODS

Commercial chickens of broiler size in the form of eviscerated carcasses in polyethylene bags or as tray-packed "cut-up" poultry meat were purchased from several retail stores in each of three widely separated cities known to be receiving poultry from different production areas in Canada. A total of 100 each of CTC-treated and nontreated specimens were obtained over an interval of several months to accommodate to seasonal differences to temperature and to allow for a broadly representative sampling. Each specimen was divided equally by aseptic methods. One-half was examined immediately; the second portion was stored in a refrigerator at from 5 to 7 C for 5 days, the respective portions being considered to represent "fresh" and "spoiling" specimens. Repulsive odours and surface slime were often present among the latter specimens.

An aliquot of 500 g from each halved specimen was cut into small pieces and added to 500 ml of sterile distilled water contained in a sterile screw-capped Waring Blendor vessel and shaken vigorously for 10 min in a mechanical shaker. Progressive dilutions of the wash-water were prepared from treated and nontreated specimens in the fresh and spoiling state, and used for the inoculation of specific media for the determinations described below. Numerical estimates of different microbial categories were carried out by customary plate-count procedures, using the following specific media and incubation times and temperatures as noted: (a) Mesophilic plate count: tryptone glucose extract-agar (Difco),[2] 35 C for 48 hr; (b) "psychrophilic" plate count: tryptone glucose extract-agar, 5 to 7 C for 48 hr; (c) coliforms: violet red bile agar (Difco), 37 C for 24 hr; (d) staphylococci: mannitol-salt agar (Difco), 37 C for 48 hr, followed by determination of the proportion that were coagulase positive by the tube test using rabbit plasma (Difco) as the test substrate; (e) enterococci: enterococcus confirmatory agar, 45 C for 72 hr (Ross and Thatcher, 1958); (f) yeasts: Skinner's (Skinner, 1947) 1 per cent peptone beef extract agar with added tartaric acid and glucose. Replicate specimens in this medium were incubated for 5 days at each of the three temperatures, 22, 5 to 7, and 35 C, to allow the respective enumeration of saprophytic yeasts of mesophilic and of psychophilic properties and of potentially pathogenic yeasts.

The proportion of coliforms that were *E. coli* was determined by transfer of inocula from characteristic colonies on violet red bile plates to EC broth (Difco) which was incubated at 44.5 C in a water bath, followed by smearing plates of eosin methylene blue agar with specimens from gas-positive tubes and noting the appropriate reactions. Some possible degree of error from this last step is recognised, but when 12 cultures originating in this way were all shown to be *E. coli* type I by IMViC tests, the potential error was considered acceptable for the present purposes, and the method saved time as compared with confirmed most probable number (MPN) procedures for both coliforms and *E. coli*.

Examination for the presence of salmonellae was carried out by first centrifuging the residual poultry wash-water (about 450 ml) from each specimen at 4500 rpm for 20 min to separate bacteria from soluble food materials (Silliker and Taylor, 1958). After discarding the supernatant, the remaining sediment (1 to 2 ml) was suspended in 99 ml of selenite F broth containing cystine (North and Bartram, 1953) at the rate of 10 mg per L and incubated for 18 hr at 37 C. The selenite preparation was used to streak plates of

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Kauffmann brilliant green, bismuth sulphite, and SS agars (all Difco products). After incubation at 37 C for 24 hr, representative colonies of those resembling *Salmonella* were used to inoculate tubes of triple-sugar-iron and urea agars, and specimens with appropriate reactions were purified on plates of MacConkey agar and tested on the following sequence of differential media: Simmon's citrate agar, gelatin, tryptone broth for the production of indole, and the broths methyl red-Voges-Proskauer, dulcitol, lactose, salicin, and sucrose. Appropriate cultures were tested with *Salmonella* polyvalent-O diagnostic sera before being typed at the Laboratory of Hygiene, Department of National Health and Welfare, through the courtesy of Dr. E. T. Bynoe. Tests for *Salmonella* were made from 90 nontreated and 80 CTC-treated carcasses.

The sensitivity to CTC of all isolates of *Salmonella* and of *Staphylococcus* was determined by establishing the maximal concentration of CTC present in 10 ml of nutrient broth that would allow growth in 48 hr at 37 C, using 1 loopful of an 18-hr broth culture as inoculum. An initial series of concentrations of CTC was established ranging from 7 to 116 ppm at 2-fold intervals, the lower concentration being chosen because this is the maximal level permitted in or upon poultry. Cultures of *Salmonella* failing to grow in the presence of 7 ppm CTC were retested in a series of descending values to 0.10 ppm.

To estimate the proportion of the respective populations of mesophilic and psychrophilic bacteria and of yeasts that were resistant to levels of CTC in excess of the concentrations permitted in poultry, the series of plates designed for enumeration of the various microbial categories was duplicated but with the addition to the respective media of CTC at a concentration of 7 ppm.

Colonies representative of the numerically dominant bacteria present on plates at terminal dilutions from all media used for enumerating purposes were purified prior to determination of their individual resistance to CTC. Totals of 1,070 colonies from CTC-treated and 370 from nontreated specimens were isolated in this way. The resistance to CTC was determined for each culture by the tube method, incubation being at the

TABLE 1

Content of specific microbial groups in market poultry, treated and nontreated with chlortetracycline (CTC) and in the "fresh" and "spoiling" condition

Microorganisms	Microbial Values: No. per Gram					
	CTC-treated (100 specimens)			No CTC (100 specimens)		
	Min.	Max.	Average	Min.	Max.	Average
SPC* (mesophilic):						
"Fresh"	1,500	590,000	18,000	1,500	210,000	31,000
"Spoiling"	70,000	580,000,000	6,656,000	1,100,000	62,000,000	8,894,000
SPC (psychrophilic):						
"Fresh"	3,500	4,700,000	229,000	260,000	7,800,000	853,000
"Spoiling"	630,000	780,000,000	120,375,000	15,000,000	515,000,000	124,620,000
Staphylococci (coagulase-positive):						
"Fresh"	0	420	54	0	32	8
"Spoiling"	0	2,240	80	0	135	18
Enterococci:						
"Fresh"	0	420	48	0	2,350	263
"Spoiling"	0	6,100	622	0	11,200	992
Coliforms:						
"Fresh"	0	4,700	375	50	2,000	453
"Spoiling"	0	680,000	77,000	5,000	510,000	133,000
Escherichia coli:						
"Fresh"	0	3,760	300	64	1,600	362
"Spoiling"	0	544,000	61,000	4,000	408,000	106,000
Mesophilic yeasts:						
"Fresh"	120	8,700	1,382	0	7,100	774
"Spoiling"	300	1,440,000	116,900	0	39,500	6,800
Psychrophilic yeasts:						
"Fresh"	150	17,000	2,802	0	1,250	315
"Spoiling"	200	710,000	119,700	0	17,500	4,275
Potentially pathogenic yeasts:						
"Fresh"	0†	0	0	0	0	0
"Spoiling"	0	0	0	0	0	0

* Standard plate count.

† Absent from triplicate plates at 1:10 dilution.

respective temperatures used during initial isolation of each culture. A selection of 170 cultures that proved to be the more resistant to CTC within each source category (including representatives of mesophilic and psychrophilic isolates from fresh and spoiling, treated and nontreated poultry) were subjected to taxonomic determinative tests. The determinations made initially were: Gram reaction; motility (a) hanging drop method, (b) semi-solid "motility" agar; flagella stain (Gray's method); the production of acid and/or gas from glucose and lactose; the reduction of nitrate to nitrite, liquefaction of gelatin, and litmus-milk reaction, all in accord with standard methods in the *Manual of Microbiological Methods* (SAB, 1957).

With the results from these determinations, classification to family and for the most part to genus was possible with the aid of *Bergey's Manual* (Breed, Murray, and Smith, 1957). Isolates with identical properties at this stage were considered to be of the same species. Single representatives of each "species" were then subjected to further determinative tests as shown to be desirable from the expanded description of species within *Bergey's Manual*. Species names within most genera are not offered since comparative studies with authentic type cultures were not undertaken. Groups of organisms that seemed to be of the same species are listed by index number.

Results

The numbers of microorganisms recovered from poultry wash-water are shown in table 1. The data express the respective average values from all specimens in fresh and in spoiling condition and treated and not-treated with CTC.

The data in table 1 show that the numerically predominant bacteria both in fresh and spoiled poultry are of the psychrophilic group. The CTC treated poultry in "fresh" condition contained psychrophiles and mesophiles in the ratio of 12:1. In the absence of CTC this ratio was 28:1. The values for both categories of bacteria were greater in the untreated specimens, the greatest difference being a 4-fold reduction of psychrophiles in the presence of CTC.

CTC was associated with an increase in the numbers of yeasts. The psychrophilic yeasts of the CTC-treated specimens showed average increments of 9-fold in the fresh condition and 28-fold in the spoiling condition, as compared with nontreated specimens. No yeasts that would grow at 35 C were recovered, from which it is concluded that yeasts pathogenic to man were not present in significant numbers.

The data expressing the prevalence of bacteria of possible public health significance are also shown in table 1. Staphylococci were present in relatively low numbers and increased very little during storage, either in the presence or absence of CTC. The maximal number present in a treated specimen in "fresh" condition was 420 per g. The stored half of the same carcass at time of spoilage contained 2,240 per g.

Enterococci were common, but during storage they multiplied slowly. The average values increased during the 5-day storage period from 48 to 622 per g in the presence of CTC; from 263 to 992 per g in the absence of CTC.

Coliforms multiplied substantially during storage. Initial average numbers were 375 and 453 per g in CTC-treated and nontreated specimens, respectively. Comparable values were 77,000 and 133,000 per g after storage. The numerical estimate for coliforms was equivalent to 2.1 per cent of the total population of mesophilic bacteria in treated fresh specimens, and 1.4 per cent in nontreated specimens. After spoilage, this proportion remained similar at 1.2 per cent in both treated and untreated specimens. Eighty per cent of all coliform colonies selected from violet red bile agar were shown to be *E. coli*.

As shown in table 2, from carcasses in the "fresh" condition, *Salmonella* was isolated from 14 nontreated specimens and from only 1 treated specimen. (Two serotypes were isolated from a single nontreated carcass.) Recovery of *Salmonella* in the spoiled condition was limited to two untreated carcasses. Four different serotypes were recognised: *S. gallinarum*, *S. typhimurium*, *S. oranienburg*, *S. indiana*. It is of interest to note that the isolation of *S. indiana* from 4 carcasses represents the first record of the presence of this serotype in Canada.

All *Salmonella* isolates grew in broth containing 0.21 ppm CTC, but none grew at a concentration of 0.43 ppm.

The comparative plate count values for treated and nontreated specimens in the "fresh" and "spoiling" condition using standard media and media containing 7 ppm CTC are listed in table 3. The tabulated percentages of the respective bacterial populations resistant to 7 ppm demonstrate a progressive selective

TABLE 2

Isolation of Salmonella from market-poultry treated and non-treated with chlortetracycline (CTC)

Salmonella Serotypes Isolated	No. of Specimens Yielding *Salmonella* (from 90 Treated, 80 Nontreated Chickens)			
	CTC-treated		No CTC	
	"Fresh"	"Spoiling"	"Fresh"	"Spoiling"
S. gallinarum	0	0	1	0
S. typhimurium	0	0	4	0
S. oranienburg	1	0	5	1
S. indiana	0	0	4	1
Total recovery	1	0	14	2

action by the CTC in favouring the multiplication of CTC-resistant bacteria. In the fresh condition, 6 per cent of the total mesophilic plate count was resistant to CTC in both the treated and nontreated specimens. In the spoiled condition, these respective values were 19 and 4.2 per cent. The proportion of resistant "psychrophilic" bacteria recovered from treated specimens increased from 18 to 35 per cent during the 5-day storage period, the average numbers at the two stages being, respectively, 42×10^3 and 43×10^6. The resistant proportion of the psychrophilic population among the nontreated specimens was 0.7 per cent when fresh and 1.8 per cent when spoiling.

The comparative resistance of micrococci, coagulase positive staphylococci, enterococci, *E. coli*, and of the numerically dominant organisms recovered from an agar medium containing 7 ppm CTC is shown by the data in table 4. The data express the numbers of organisms among those tested that were able to grow in the presence of progressive levels of CTC.

Resistance among the micrococci and staphylococci from poultry of either treatment was limited to 7 ppm, a total of 5 cultures from the 130 tested having been found at this level. Most of the enterococci from treated specimens, whether in the fresh or spoiling state were resistant to 28 ppm. Among the nontreated specimens a smaller proportion of these organisms were resistant and to a maximal value of 14 ppm. The frequency of resistance among isolates of *E. coli* arising from treated poultry was noteworthy. Only 6 of 110 cultures of *E. coli* from "fresh" treated specimens were resistant to 28 ppm; 69 of 78 were resistant to this level at time of spoilage. From spoiled nontreated specimens only 1 culture of the 20 tested was resistant to 7 ppm.

The data in table 4 also shows a selectivity for resistant strains of both mesophilic and psychrophilic spoilage organisms, although this seems to occur also, but to a less degree, in the absence of CTC. From the "spoiled" state, 1 of 24 cultures tested from nontreated specimens was resistant to 112 ppm; 10 of 75 from

TABLE 3

Comparative numbers of chlortetracycline (CTC)-resistant spoilage bacteria on CTC-treated and nontreated market poultry, in the "fresh" and "spoiling" condition

Bacterial Category	Bacterial Content: Average No. per Gram ($\times 10^3$)					
	CTC-treated			No CTC		
	Total plate count	Resistant* plate count	Per cent resistant	Total plate count	Resistant plate count	Per cent resistant
Mesophilic plate count: "fresh"	17.6	1.1	6	31.1	1.8	6
Mesophilic plate count: "spoiling"	6,656	1,317	19	8,894	377	4.2
Psychrophilic plate count: "fresh"	229	42	18	853	6.9	0.7
Psychrophilic plate count: "spoiling"	120,375	43,343	35	124,620	2,321	1.8

* Resistant = able to grow on agar containing 7 ppm CTC.

TABLE 4

Comparative sensitivities to chlortetracycline (CTC) of bacteria isolated from CTC-treated and nontreated poultry

Microbial Category and Source	No. of Cultures Showing Growth in Broth Containing Specific Levels of CTC (ppm)											
	CTC-treated						No CTC					
	0	7	14	28	56	112	0	7	14	28	56	112
Micrococci ("fresh"*)	87	43	0	0	0	0	30	0	0	0	0	0
Staphylococcus aureus ("fresh")	12	1	0	0	0	0	1	0	0	0	0	0
Enterococci ("fresh")	52	48	48	48	0	0	25	20	20	0	0	0
Enterococci ("spoiled")	20	20	20	20	0	0	12	11	11	0	0	0
Escherichia coli ("fresh")	110	35	35	6	0	0	30	2	0	0	0	0
Escherichia coli ("spoiled")	78	69	69	69	0	0	20	1	0	0	0	0
SPC† 35 C ("fresh")	96	75	62	12	12	12	24	7	6	0	0	0
SPC 35 C ("spoiled")	96	64	56	24	24	24	24	10	10	8	1	1
SPC 5–7 C, ("fresh")	62	7	5	5	5	3	24	14	10	0	0	0
SPC 5–7 C, ("spoiled")	75	25	20	15	10	10	24	16	8	5	2	1
Yeasts, 5–7 C ("fresh")	50	50	50	50	50	50	50	50	50	50	50	50
Yeasts, 5–7 C ("spoiled")	50	50	50	50	50	50	50	50	50	50	50	50

* "Fresh" and "spoiled" refer to the respective conditions of the poultry at time of examination.

† SPC refers to numerically dominant bacteria from standard plate count media containing 7ppm CTC.

treated specimens were resistant to this level. All of the 200 yeast cultures tested were resistant to greater than 112 ppm.

Table 5 lists the genera and the numbers of the various "species" groupings represented among the selected CTC-resistant spoilage bacteria, and records also the maximal level of CTC tolerated within each "species." Nine species-groups were allocated to the genus *Pseudomonas*, three to *Alcaligenes*, eight to *Achromobacter*, three to *Flavobacterium*. The rest were common members of *Escherichia* and *Aerobacter*.

The percentages of the numerically dominant resistant organisms present among spoiled, nontreated specimens and allocated to the various genera were: *Pseudomonas* 62 per cent, *Achromobacter* 27 per cent, *Alcaligenes* 11 per cent. The comparable values from treated specimens were 36, 59, and 2.6 per cent, respectively, suggesting that the CTC treatment exerted a selective effect favouring the multiplication of *Achromobacter* during storage. The specific resistance data in table 5 show that the highest degree of resistance within the cultures of a particular "species" occurred in isolates from the CTC-treated poultry among 12 of the species, and from nontreated specimens in 2

TABLE 5

Chlortetracycline (CTC)-resistant spoilage bacteria isolated from commercial poultry treated and nontreated with chlortetracycline

Genera and "Species" Groups	No. of Cultures Isolated from Specific Sources				Maximum Resistance (in ppm Tolerated)	
	CTC-treated		Nontreated		CTC-treated	Non-treated
	Fresh	Spoiled	Fresh	Spoiled		
Pseudomonas "species":						
1	1	—	3	5	28	56
2	2	1	2	—	28	28
3	—	—	1	7	—	56
4	9	4	—	3	28	112
5	1	1	—	—	28	—
6	2	3	2	—	56	14
7	1	5	5	7	112	28
8	2	—	—	—	14	—
9	—	—	—	1	—	28
Alcaligenes "species":						
1	2	—	5	2	14	14
2	—	1	—	2	56	28
3	1	—	1	—	56	14
Achromobacter "species":						
1	1	3	—	1	112	28
2	4	6	—	2	112	28
3	—	3	—	1	112	28
4	3	1	1	—	56	7
5	5	9	1	6	112	28
6	—	1	2	—	28	14
7	—	—	—	1	—	112
8	3	—	—	—	112	—
Flavobacterium "species":						
1	—	—	2	—	—	7
2	—	—	1	—	—	7
3	—	—	2	—	—	14
Escherichia freundii	3	—	1	—	112	7
Escherichia coli	2	—	3	—	28	28
Aerobacter aerogenes	—	1	3	—	56	14
Aerobacter cloacae	—	—	2	—	—	28
Total Determinations						
Pseudomonas	18 (43)*	14 (36)	13 (35)	23 (62)		
Alcaligenes	3 (7)	1 (2.6)	6 (16)	4 (11)		
Achromobacter	16 (38)	23 (59)	4 (11)	10 (27)		
Flavobacterium	—	—	5 (13)	—		
Escherichia	5 (12)	—	4 (11)	—		
Aerobacter	—	1 (2.6)	5 (13)	—		
	42	39	37	37		

* Figures in parentheses are percentages of the total from each source.

instances. This points both to selection of resistant organisms and an increase in the level of resistance as a result of treatment, but shows also that highly resistant organisms were present in the absence of CTC.

Discussion

It is recognised that the conclusions to be deduced from the present study might be subject to modification if a more extensive sampling had been practicable.

The data presented in this paper reveal no evidence to suggest that the commercial use of CTC for the preservation of poultry under Canadian conditions has contributed in any way to increased risk of food-poisoning or of exposure to pathogenic yeasts. Njoku-Obi *et al.* (1957) reported the presence of a possible pathogen, *Candida parapsilosis*, from CTC-treated poultry, but our negative findings are in accord with Walker and Ayres (1959). Salmonellae were recovered from nontreated and treated chickens in the ratio of 14:1, respectively, and none of the 15 *Salmonella* isolates were resistant to CTC at the level of 7 ppm. The fact that salmonellae were recovered much more frequently from poultry in the fresh as compared with the spoiling condition whether CTC-treated or not, suggests that poultry under refrigerated conditions do not offer an environment favourable to multiplication of *Salmonella*. The psychrophilic bacteria and yeasts are destined to become dominant.

Because coliforms multiplied substantially, the argument might be advanced that the method used failed to recover *Salmonella* from spoiled specimens because of the presence of large numbers of coliforms, a familiar difficulty. However, coliforms were present in similar numbers in treated and nontreated specimens. Hence, it seems probable that under normal conditions of Canadian marketing of CTC-treated poultry (packed in chipped ice during transport and continuously refrigerated in retail stores) the observed reduction in frequency of isolation of *Salmonella* from CTC-treated chickens is due either to competitive activity of the selectively favoured flora or to destruction of the susceptible *Salmonella* by the antibiotic, *per se*. It would appear that the emergence of CTC-resistant *Salmonella* (Huey and Edwards, 1958) has not occurred among Canadian poultry with sufficient frequency for its detection by the sampling reported herein.

No significant change was demonstrated in the numerical prevalence of coliforms, enterococci, or of coagulase positive staphylococci as a response to the use of CTC.

CTC-treatment, however, was associated with a higher proportion of resistant enterococci and of *E. coli* and with an increased level of resistance among both organisms. Elliott and Barnes (1959) have shown the selection of antibiotic-resistant serotypes of *Streptococcus faecalis* in response to dietary CTC, but Goldberg, Goodman, and Lanning (1959) state that low-level feeding of CTC to laboratory mice did "not cause detectable emergence of resistance by coliform bacteria." Addition of CTC to an enclosed enteric population *in vivo* may not be comparable in effect to its superficial application, but resistant coliforms were present in untreated poultry, which may have resulted from the common practice of dietary use of CTC for poultry or from the establishment of these organisms in processing plants that used CTC intermittently (some plants use CTC only for poultry offered for "week-end" sales). Our data point to a selective favouring of resistant strains during storage of treated poultry in accord with the opinion of Walker and Ayres (1958) and as previously postulated by Morrell and Thatcher (1957) and by Thatcher (1958).

The observation of Gale and Hall (1959) that "submaximal levels" of CTC "markedly inhibited growth of psychrophilic pseudomonads" seems to be confirmed in part by our finding that the resistant pseudomonads, numerically dominant in "spoiled" untreated poultry, were less numerous than resistant *Achromobacter* species in their CTC-treated counterparts.

Nagel *et al.* (1960) did not find such an effect associated with the use of oxytetracycline as a preservative for poultry. Ng *et al.* (1957) report the prevalence of resistant spoilage bacteria upon chickens processed in the presence of CTC.

At time of spoilage the average increment of psychrophilic yeasts in the presence of CTC was 50-fold greater than in the untreated specimens.

The differences observed between the average values for the various microbial categories enumerated from "fresh" and "spoiled" poultry are probably minimal due to the fact that some of the so-called "fresh" specimens were approaching spoilage at time of purchase.

Summary

A comparative bacteriological examination of poultry treated and not treated with chlortetracycline (CTC) and purchased retail in Canada established a selective development during storage of CTC-resistant spoilage organisms and particularly of psychrophilic bacteria and of yeasts.

No data to indicate an increased health hazard as a result of the use of CTC as a poultry preservative were obtained. No evidence was revealed for modification of hazard due to the presence of staphylococci, enterococci, coliforms, or pathogenic yeasts. The recovery of *Salmonella* was markedly reduced in the presence of CTC. Salmonellae resistant to greater than 7 ppm CTC were not found among the 15 cultures isolated from a total of 170 carcasses.

REFERENCES

Breed, R. S., Murray, E. G. D., and Smith, N. R. 1957 *Bergey's manual of determinative bacteriology*, 7th ed. Williams & Wilkins Co., Baltimore, Maryland.

ELLIOTT, S. D. AND BARNES, ELLA M. 1959 Changes in serological type and antibiotic resistance of Lancefield group D streptococci in chickens receiving dietary chlortetracycline. J. Gen. Microbiol., **20,** 426–433.

GALE, G. O. AND HALL, R. H. 1959 The influence of submaximal antibiotic levels on the growth of chlortetracycline-resistant bacteria. In *Antibiotics annual 1958–1959*, pp. 1040–1046. Medical Encyclopedia, Inc., New York, New York.

GOLDBERG, H. S., GOODMAN, R. N., AND LANNING, B. 1959 Low-level, long-term feeding of chlortetracycline and the emergence of antibiotic-resistant enteric bacteria. In *Antibiotics annual 1958–1959*, pp. 930–934. Medical Encyclopedia, Inc., New York, New York.

HUEY, C. R. AND EDWARDS, P. R. Resistance of *Salmonella typhimurium* to tetracyclines. Proc. Soc. Expl. Biol. Med., **97,** 550–551, 1958.

MORRELL, C. A. AND THATCHER, F. S. 1957 Antibiotics in foods. Food Drug Cosmetic Law J., **12,** 477–491.

NAGEL, C. W., SIMPSON, K. L., NG, H., VAUGHN, R. H., AND STEWART, G. F. 1960 Microorganisms associated with spoilage of refrigerated poultry. Food Technol., **14,** 21–23.

NG, H., VAUGHN, R. H., STEWART, G. F., NAGEL, C. W., AND SIMPSON, K. L. 1957 Antibiotics in poultry meat preservation: development of resistance among spoilage organisms. Appl. Microbiol., **5,** 331–333.

NJOKU-OBI, A. N., SPENCER, J. V., SAUTER, E. A., AND EKLUND, M. W. 1957 A study of the fungal flora of spoiled chlortetracycline treated chicken meat. Appl. Microbiol., **5,** 319–321.

NORTH, W. R. AND BARTRAM, M. T. 1953 The efficiency of selenite broth of different compositions in the isolation of *Salmonella*. Appl. Microbiol., **1,** 130–134.

ROSS, A. D. AND THATCHER, F. S. 1958 Bacteriological content of marketed precooked frozen foods in relation to public health. Food Technol., **22,** 369–371, 1958.

SILLIKER, J. H. AND TAYLOR, W. I. 1958 Isolation of salmonellae from food samples II. The effect of added food samples upon the performance of enrichment broths. Appl. Microbiol., **6,** 228–232.

SKINNER, C. E. 1947 The yeast-like fungi: *Candida* and *Brettanomyces*. Bacteriol. Rev., **11,** 227–274.

Society of American Bacteriologists 1957 *Manual of microbiological methods*. McGraw-Hill Book Co., Inc., New York, New York.

THATCHER, F. S. 1958 Antibiotics in foods: a review of some public health aspects. Can. J. Public Health, **49,** 58–72.

WALKER, H. W. AND AYRES, J. C. 1958 Antibiotic residuals and microbial resistance in poultry treated with tetracyclines. Food Research, **23,** 525–531.

WALKER, H. W. AND AYRES, J. C. 1959 Characteristics of yeast isolated from processed poultry and the influence of tetracyclines on their growth. Appl. Microbiol., **7,** 251–255.

A Comparative Procedure for Evaluating Antimicrobial Activity of Gaseous Agents[1]

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Received for publication April 20, 1960

The increasing use of gaseous sterilization (Nordgren, 1939; Salle and Korzenovsky, 1942; Ingram and Heines, 1949; Phillips, 1949; Kolb and Schneiter, 1950; Logrippo *et al.*, 1955; Newman, Colwell, and Jameson, 1955; Hoffman and Warshowsky, 1958) has called the attention to the need for a uniform, reproducible procedure to investigate the antimicrobial activity of gaseous agents. The classical methods for testing disinfectant activity are designed for aqueous agents and thus do not give adequate consideration to some of the factors involved in gas exposure techniques. The criterion used for measuring the effect of a gas or gases on organisms should be a reproducible function of the concentration and duration of exposure.

Apparatus to study this has been designed and tested. The technique requires exposing a thin layer of organisms collected on Millipore filter paper to the gaseous agent, transferring the bacteria to the growth medium, and determining the growth lag by change in optical density.

MATERIALS AND METHODS

Apparatus for exposure of organisms to gases. Figure 1 is a diagrammatic drawing of an all glass chamber for single filter exposures. It is made from a Pyrex Petri dish (100 by 15 mm) with a 7 mm outside diameter tubing fused into the base. A 12/5 socket connects to the gas line and an equal orifice quadruple outlet, blown inside the base, provides uniform dispersion of the gases. Imperfections in fit of the Petri dishes suffice to provide an exit for the gases.

Figure 2 is a diagram showing a cross section of the multiple (4 filters) exposure chamber. It is con-

[1] This work was supported by a research contract with the Air Pollution Medical Program, Public Health Service, U. S. Department of Health, Education and Welfare. A preliminary report is as presented at the 59th general meeting of the Society of American Bacteriologists, St. Louis, Missouri, May 1959.