○ 疾病罹患による喪失労働日（disability adjusted life year: DALY）その他

国内からの報告はなく、海外の報告例としては、アメリカによるサルモネラの推定年間経済損失12～15億ドルに匹敵すると考えられている（Koopmans et al , 2002）。

3． 食品製造、加工、流通と摂取

* リスクマネジメントに関与し、影響を与え得る媒介食品の特性：

生食用のカキは国内の条件を満たす特定海域で養殖されている。調理用（加熱用？）はそれ以外の海域でも養殖を行うことが出来る。また最近では、需要の増加に伴い中国や韓国からの調理用カキの輸入が増えている。これらのカキが混在して流通していることが消費者におけるカキの生食の実態をつかむ上での問題点になるかもしれない。また、生食の基準は、現在食品中の大腸菌数のみで決められており、ウイルスに関しては基準が設定されていない。現段階で考えうるマネジメント・オプションとして挙げている、ワッシュ・アウト時間の調整は、カキの実入りと反比例の関係にあることが知られており、仮りに有効性が認められオプションとして採用する場合、カキの品質と安全の両者間のバランスを考慮することが必要である。

* 媒介食品の微生物学的安全性に影響を与える要素を含めた、生産から消費までの連続過程（一次生産過程、加工過程、流通・輸送、貯蔵・保存、調理など）の解説：

1． 種カキの汚染：生産海域の海水の汚染状況

2． カキの養殖と収穫：養殖漁場の海水の汚染状況、海水温、潮流、塩濃度などが影響すると考えられている。貝類の濃縮機構は重要な要素であると報告されている。それと関連して、ワッシュ・アウト期間の周囲環境がウイルス濃度の希釈に大きく関与する。現実的には相当量のウイルス減少することが出来るが、20時間程度では完全に除去できない。

3． 加工・袋詰・市場：この部分は、殻つきと剥き身に分けて見る必要がある。作業従事者の健康管理と衛生的に作業が行われることと、洗浄、袋詰に用いられる水の種類と質がこの段階で交差汚染に関与するもっとも重要な要素となる。

4． 流通、再パッケージングおよび小売：生食用、調理用に分けた消費者に解り易い表示方法と産地、ロット、生産者表示等の統一による製品管理が必要である。再パッケージングに関しては、上記第3項を参照とし、特に無症状の感染者による交差汚染に十分な注意を払う必要がある。

5． 外食産業（レストラン、ケータリング、仕出し）、給食施設および消費者：調理と下準備における取り扱いの方法と、調理従事者からの交差汚染が重要な要素となる。

* リスクに関して現在知られていること、例えば媒介食品の生産、加工、流通と消費者のハンドリングに関連してどの様にしてリスクが発生し、誰に影響を及ぼすか：

1． 培養海域の海水のノロウイルスによる汚染のため、漁穫時にカキおよび二枚貝が汚染

されている。
2．水揚げ直後の剥き身作業、袋詰め作業と市場における操作時に交差汚染されている可能性が考えられる。
3．流通過程における増殖は考えにくいが、袋詰めもしくは箱付めされているための梱包内交差汚染の可能性があり、個々のカキもしくは二枚貝内のウイルス濃度、汚染頻度において影響がある。
4．調理施設における交差汚染が摂取時の頻度や濃度へ大きく影響している可能性がある。

* 既存のリスクマネジメントの効果の範囲と有効性についての以下を含む要約：食品の生産と加工に関する食品衛生規範・基準、教育プログラムやセミナー、（ワクチンなどを用いた）介入型公衆衛生プログラム：

現在のカキの品質管理は食品衛生法に基づき、大腸菌数、腸内細菌群数によって管理されている。一部の生産者は最近のノロウイルスの感染の増加に対して、独自の基準と品質管理のガイドラインを作り、出荷前のサンプリングでRT-PCR法にて陽性となった時には出荷を見合すなどの方法を取っているが、サンプリングの代表性、妥当性および出荷見合わせの有効性は確認されていない。また、ノロウイルス症例の報告が見られる時期に限り、養殖海域の海水調査も行っているが、カキ、養殖海域どちらに対するサーベイランス・システムも確立されていない。カキは同じ海域でも個体によりウイルス汚染は多様であり、筏のどの地点を選ぶのか、個数を幾つにするべきかの検討が必要である。また海域では測定する海の海水をどの地点を選択すべきかの問題がある。降雨量が多いときには上層部が、海が荒れたときには下層から汚染されるのでそれらを総合的に行うべきである。

カキの生食に対する危険に関する広報は一部季節、地域により行われているが、昨今の症例の増加を鑑みると、現在までのところ大きく公衆の食習慣へ影響を与えるところまで行っていない。厚生労働省は、ノロウイルスに関するQ&Aをインターネット上で公開し、国民への啓発、不安解消に努めている。

4．その他のリスクプロファイル項目

* 当該病原体における食中毒の新規発生数の地域差

日本全国で発生している。

* 当該食品、もしくは加工食品の輸出入の状況（交易範囲、輸出入量）：

日本はカキの生食に関して世界でも有数の消費国であるが、最近までは国内産でそのほとんどを賄ってきた。消費量の増大に伴い、国内産の生食へのシフトが更に進むとともに、一部輸入カキが生食へ用いられるようになってきている。今後、この割合は増えると思われ、細菌のみならずウイルスの基準も必要となると思われる。

* この問題とリスクに関する世論の認知度：

近年のマスコミにより報道された数多くのノロウイルスによる集団発生の事例から、国民は海産物特にカキに代表される貝類の生食による冬期の感染の危険は周知していると考えられるが、どの程度の調理により、どの程度感染が回避されるかについての情報は不足している。冬期のカキの生食および軽く火を通した食習慣は一般的なものであり、指摘されたリスクの大きさは個々人のレベルで明確に理解されていない。一般的心情として、未知のリスクは誇大にもしくは過小に評価しがちで、従来の行動様式を正当化し、保全する為にこのケースでは過小に評価しているのではないかと考えられる。

* Codex に準じたマネジメント・ガイダンスを確立することにより、公衆衛生および経済上、考え得る影響：

実際のリスクの大きさと関与する因子を明確に示すことにより、国民は取るべき行動と自己責任の範囲を知ることが出来る。ガイダンスに従って、広報活動を行うことにより、よりリスクの高い集団に対して、重篤な症状を引き起こす危険回避の手段を与えることが出来る。現在、集団発生のたびに大きな経済的打撃を受けつつも、有効な対策指針を持たないカキ業界への、不要な試験を省き、必要な対策のみに投資することにより出荷停止を免れることによる、経済的インパクトは大きいと考えられる。同時に、他の後遺症が残るもしくは死亡例の出るような細菌性の食中毒に比べるとあくまでも小さいが、個々人における下痢症による経済活動の損失を防ぎ、その累積により大きな経済損失を防止することができる。阿部らの試算に単純に 2001 年の罹患者数を乗じて、今後の食中毒患者数の増加とそれによる賠償額を求めると(食中毒患者全員に対し何らかの賠償が必要として)、最高年間約 1 億 1400 万円の経済損失を免れることが見込める (Abe et al, 2000)。

5． リスクアセスメントの必要性とリスクアセッサーへの質問提起

* リスクプロファイルに基づき、微生物学的リスクアセスメントがマネージャー側の必要とする情報の解析を十分に行い、希望する結果・内容の提供用件を満たす手段として適当であるかに対する見解と、計画しているリスクアセスメントによって求めている結果に対して、現況で想定できる提言および、それが実際の施策にどのように反映しえるかについての検討：

食中毒統計から、カキの生食と不十分な加熱調理での摂取が、原因食が明らかになっている食中毒事例のうち大きな割合を占めていることは明らかである。これによるリスクは、現在までに解っている基礎実験データより、ワッシュ・アウト期間に何らかの基準を設けることにより減少できる可能性が示唆されている。しかし、その効果の程度は不明であり、既に行われている購入から摂食までの時間の短縮の推奨や、保蔵状態のなど管理の強化との比較検討が必要である。これを科学的に評価するためには、微生物学的リスクアセスメントは不可欠である。さらに、現在のところ SRSV/NLV による下痢症発生のリスクの大きさは定量的に明確に示されておらず、検出技能の向上によってウイルスが同定報告される様になったこともあり、一般の関心も高まり、生産者、消費者双方から新しい基準の設定の

希望が出てきている。微生物学的リスクアセスメントの結果からリスクの大きさの程度、微生物学的新基準、生食用海産物の養殖や取り扱いに関するガイドラインおよび患者数減少のための対策と食品以外の原因によるノロウイルス患者の実態把握の方法などへ対する示唆、提言が期待できる。また、下水処理場におけるウイルス除去効率を上げる効果についても科学的に推定ができる。

しかし、カキ汚染は乳幼児のノロウイルス排泄状況、下水処理場での処理状況、降雨量、海域での海流、養殖海域での筏の河川水の受ける影響等を総合的に研究しなければ実態の究明は困難である。

* 仮にリスクアセスメントが必要であることが確認されたとして、マネージャー側からアセッサーへ問いかける初期の質問事項及び解析を希望する事項：

ノロウイルスによる真の年間罹患者数および、集団発生における感染経路と原因の内わけが現行のシステムで十分に把握されているか？

上に挙げたマネジメントオプションの効果と効率の比較。

1） カキの十分な加熱調理の指導
2） 養殖海域、養殖過程の産物、出荷時の産物の微生物学的基準の変更および強化
3） UV 殺菌水等による出荷前の洗浄
4） 出荷前にワッシュ・アウト期間を設けることの有効性
5） 下水処理場におけるウイルス除去効率を上げることの有効性

6． 現在の入手可能な情報と、不足している知見および情報

* この病原体・媒介食品の組み合わせに対する、既存の国家単位のリスクアセスメントの存在：

少ない。絶対的な情報量の不足により完全ではないものの、欧州共同体より国際的リスクアセスメントの枠組みに従ったリスクの検討報告がだされている (European Commission, 2002)。

* リスクアセスメントを実行することも含め、リスクマネジメント活動を促進するその他の関連した科学的知見やデータの存在：

最近の知見によれば、カキおよび養殖の二枚貝に関しては、公衆のリスクを減少し得る、生産者側とも合意しあえる「ワッシュ・アウト」期間を提示できるものと考えられる。

* Codex に準じた、リスクマネジメントのガイダンスを作成するのに役立つ情報源（研究機関、官製情報、個人研究者など）と科学者：

厚生労働省----------食中毒統計、食中毒詳報、感染症発生動向調査、病原微生物検出情報

国立感染症研究所----------ウイルス第二部（武田）、感染症情報センター

(岡部、西尾)
東京都立衛生研究所---------- (関根)
国立医薬品食品衛生研究所--------食品衛生管理部 (山本、春日)
海外----------Greg Paoli、David Vose
"Opinion on veterinary measures relating to public health on Norwalk-like viruses", adopted on 30-31 January 2002 by European Commission, Health & Consumer Protection Directorate-General.

* リスクマネジメントを行う上で障害となり得る情報の欠如の存在領域:
1)カキにおける活性型ノロウイルス(現在組織培養が出来ないので活性の有無は知る手段が無い)の濃度もしくは分離頻度についての定量的情報量の不足
2)確立した、高感度の定量的ウイルス同定システム(RT-PCRは半定量法で、すべてのDNAを検出する為に、不活化ウイルス由来のDNAをも含めて検出する)
3)養殖条件(温度、期間、海域内配置、プランクトン発生等)の記載形式が統一されておらず、記録が不定期
4)集団発生の際の原因食材のトレースバックのシステムが不完全(バッチ、ロットの記載が義務化されていない、収穫時期の記載義務が不十分、養殖海域のどの部分からの収穫か記録がない等)
5)臨床症状の発生に必要なウイルス量が不明である。このウイルスに関するD-Rカーブがほとんど存在しない
6)ノロウイルスに関する人免疫の情報が少ない(ハイリスク・グループの存在の有無も含めて不明)。
7)加熱調理、調理手法、消毒などのノロウイルスに対する効果の情報不足
8)サーベイランスからのノロウイルス患者情報の不足(現行の感染症サーベイランスでは感染性胃腸炎の中に含まれて報告されるため、実数は不明)

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Prolonged Incubation Period of Salmonellosis Associated with Low Bacterial Doses

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ABSTRACT

In gastroenteritis outbreaks caused by *Salmonella*-contaminated lunches at elementary, junior high, and nursery schools, outbreaks with long median incubation periods (i.e., 60 to 120 h) were observed frequently between 1990 and 1999 in Japan. We analyzed epidemiological data on 185 outbreaks of *Salmonella* Enteritidis infection to study the factors underlying the long incubation period. These survey results showed that the median incubation period for *Salmonella* Enteritidis infection from contaminated school and nursery school lunches was significantly longer than that from other types of cooking facilities. In addition, we analyzed the relationship between the median incubation period and the bacterial dose ingested per person in nine outbreaks of *Salmonella* Enteritidis infection; the bacterial dose was estimated with reference to the bacterial concentration in the causative foods. A significant negative correlation between the bacterial dose ingested per person and the median incubation period is clearly shown. The time elapsed from the start of the cooking process to the consumption of school and nursery school lunches was significantly shorter than at other cooking facilities, suggesting limited bacterial growth, which in turn is thought to lead to a long incubation period.

The number of outbreaks and patients suffering from foodborne *Salmonella* infection increased from 1990 to 1999 in Japan. The number of outbreaks has tended to decrease thereafter; however, *Salmonella* is even now the main causative pathogen of foodborne disease. Therefore, taking preventive measures against foodborne *Salmonella* infection is an important food hygiene issue in Japan, as is the case for many other countries. For investigations into the foods that caused *Salmonella* infection and the facilities involved, the incubation period is critical because it usually determines the data and time length of food consumption surveys in interviews with the patients.

The incubation period for *Salmonella* infection is generally reported to be 8 to 24 h, ranging from 6 to 48 h *(4)*. However, much longer incubation periods (i.e., 60 to 120 h) are frequently observed in outbreaks of gastroenteritis caused by *Salmonella* Enteritidis (SE)–contaminated school lunches of elementary and junior high schools (hereafter referred to as school lunches) and nursery schools in Japan. Oda et al. *(9)* reported that the median incubation period for foodborne infection caused by SE-contaminated nursery school lunches was as long as 108 h, and the number of ingested cells per patient was estimated to be small, 23 to 39 cells per person. Matsui et al. *(6)* reported that the median incubation period for foodborne infection caused by SE-contaminated dessert buns in Toyohasi City in 2001 was 168.0 h, and the number of cells ingested per patient was estimated to be below the detection limit by the most-probable-number (MPN) method (i.e., less than 30 cells per 100 g). Moreover, several other studies indicated that the incubation periods for some *Salmonella* infections were longer than is generally accepted *(1, 2, 8, 10, 12)*. The relationship between the prolonged incubation period and factors affecting this prolongation, including the number of cells ingested and the method of cooking, has not been analyzed thoroughly.

According to the Food Sanitation Law of Japan, local health centers of prefectures and government-designated major cities report to the prefectural governors the results of epidemiological investigations in the form of the "Food Poisoning Investigation Report." In these reports, the results of detailed investigations into items such as incubation periods, symptoms in patients, cooking methods of the causative foods, causative pathogens, and contamination routes are indicated. We collected these reports and used them to analyze the factors leading to a prolonged incubation period.

MATERIALS AND METHODS

Foodborne SE infection outbreaks used as study subjects. Among the investigation reports collected from 39 prefectures and 9 government-designated major cities in Japan from 1982 to 2002, 185 outbreaks caused by SE had data for incubation periods and microbiological tests. These outbreaks fulfilled the following requirements: (i) the number of patients in the outbreak was 10 or more, (ii) fecal cultures were positive for SE and negative for other pathogens, and (iii) causative meals or dishes were identified on the basis of microbiological tests or through interviews with the patients regarding foods eaten before the onset of illness.

Investigated items at different cooking facilities. Following the classification made by the Japanese Ministry of Health,

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Labor, and Welfare, we grouped the causative facilities as follows: elementary and junior high schools, nursery schools, restaurants, take-out food shops, hotels, and hospital and welfare facilities. We investigated patient gender and average age, median incubation period, attack rate, and the time elapsed from the start of the cooking process to consumption of the SE-contaminated food (hereafter referred to as elapsed time) for each facility group. No leftover or reserved foods had been served among the cases examined, but in one case, stored foods were served the day after. In this case, the elapsed time was calculated from the start of the preparation period.

From the nine outbreaks that provided data on SE concentrations in causative foods, the following data sets are summarized in Table 1: vehicle foods, cooking method, number of patients, attack rate, median incubation period, SE concentration in vehicle foods, amount of food intake per person, SE dose ingested per person, and food conditions before bacterial examination. The same method used in past studies could not be adopted here because testing was conducted by different municipalities. However, we were able to select nine cases for which bacterial tests were conducted at the central institute of each municipality with reliable test accuracy. In collecting these data, we selected cases with reliable data in which the preservation temperatures of foods were recorded and unclear preservation temperatures were excluded and cases in which foods had been preserved at room temperature. The laboratory techniques used for bacterial isolation and enumeration are also described in the footnotes of Table 1.

Calculation of median incubation period. In the investigation reports, each patient is tabulated in terms of incubation period (every 6 to 24 h). We selected the middle time of each incubation period range as the representative value, and the median incubation period was calculated from the representative value of the range.

Estimation of SE dose ingested per person. The SE dose ingested per person was estimated with the use of the SE concentration in the vehicle food multiplied by the amount of food intake per person. Although determining the food intake per person is difficult because of individual differences, we used the food intake per person data as determined by the schools or nursery schools that had prepared the menus firsthand. For other institutions, numerical values calculated by public health centers were used. Because pupils of schools and nursing schools are strongly encouraged to clear their plates, we calculated the intake per person by assuming that they cleared their plates. If the ingested doses were reported in the investigation reports, those values were used.

Determination of elapsed time. Elapsed time was defined as the period between the start of the cooking process, including cooking procedures such as cutting, boiling, or steaming, and the time of consumption of the cooked food. Because vegetables mixed with seafood and dressed with vinegar or other seasonings, such as salad (i.e., dishes that are not heated whole), were frequently the causative foods in school lunches, the time period from the beginning of the food preparation process to serving was surveyed, deeming it essential to take the increase in bacteria during food preparation into consideration. This information was also obtained from the investigation reports.

Statistical analysis. The relationship between the median incubation period and the ingested dose per person and between the median incubation period and the attack rate were analyzed by regression analysis. Comparison of the median incubation period, the attack rate, and elapsed time between groups was by one-way analysis of variance. The level of significance of the degree of risk was set at less than 5% ($P < 0.05$).

RESULTS

Number and gender of patients for all cooking facilities. The total number of patients whose data were used in the investigation was 27,463. Sex was identified for 27,376 (99.7%) patients, of which 14,833 (54.2%) were male and 12,543 (45.8%) female. There was no relationship between the male:female ratio and the median incubation period for different cooking facilities.

Average age of patients for different cooking facilities. Average age of patients classified according to cooking facility was 55.3 years for food prepared in hospital and welfare facilities; 40 years for food prepared in restaurants, take-out food shops, and hotels; 10.6 years for school lunches; and 4.5 years for nursery school lunches.

Median incubation period for different cooking facilities. Median incubation periods for foodborne SE infection in each kind of cooking facility are shown in Table 2. The median incubation period of the various groups ranged from 24.0 to 28.4 h for food prepared in restaurants (C), take-out food shops (D), hotels (E), and hospital and welfare facilities (F), whereas median incubation period was 80.9 h for school lunches (A) and 64.8 h for nursery school lunches (B). When the median incubation period was compared among the groups, it was significantly longer for school and nursery school lunches than for food prepared in other cooking facilities ($P < 0.01$). Furthermore, the incubation periods between food prepared in restaurants, take-out food shops, hotels, and hospital and welfare facilities were not significantly different. Figure 1 shows the distribution of the median incubation period for SE infection outbreaks. The distribution of incubation periods was broader for school and nursery school lunches than for the other groups.

Relationship between median incubation period and ingested dose per person. Table 1 shows the ingested dose per person and the median incubation period in nine outbreaks. In outbreak 1, the SE concentration in the causative food (dessert bun) was reported at less than 30 cells per 100 g by the MPN method *(6)*. On the basis of this SE concentration in the food, the SE dose ingested per person for one dessert bun was estimated to be less than 12 cells, the weight of a dessert bun being 40 g. For the analysis, 12 cells were used. In outbreak 2, the ingested dose per infant less than 4 years old and per 5-year-old child was estimated as 23 and 39 cells, respectively *(9)*. Therefore, the average value, 31 cells, was used as the ingested dose per person for this outbreak. For other outbreaks, the ingested dose per person was calculated with the use of the SE concentration in the food and the amount of food intake per person. As shown in Figure 2, a negative correlation was found between the ingested dose per person and the median incubation period ($y = -74.049 \log x + 151.6$, $r = 0.933$, $P < 0.01$).

TABLE 1. *Relationship between the median incubation period and ingested SE dose estimated with the use of bacterial quantitative data of causative foods*

Outbreak	Vehicle	Cooking method	Causative facilities	No. of patients	Attack rate (%)	Median incubation period (h)	SE concentration (g^{-1})	Food intake (g/person)	SE dose ingested (CFU/person)	Food condition
1	Dessert buns	Chestnut paste wrapped with jelly made from tapioca and then steamed	Elementary and junior high school	171	Unknown	168.0	<0.3[a]	40	12	Frozen for 77 days
2	Egg salad	Boiled macaroni, cucumber, carrot, ham, and canned corn, dressed with mayonnaise	Nursery school	42	26.9	108.0	0.78[b]	40	31	Frozen for 9 days
3	Spinach with peanut dressing	Boiled spinach, carrot, and peanuts, dressed with sauce	Elementary and junior high school	644	12.1	96.0	1.4[c]	35	49	Frozen for 7 days
4	Beef and bean sprouts with sesame dressing	Boiled beef with seasoning, boiled bean sprout, and carrots dressed with sesame oil and soy sauce	Elementary and junior high school	967	9.2	94.5	4×10[d]	22	8.8×10^2	Refrigerated
5	Boiled vegetables with sauce dressing	Boiled spinach, cabbage, bean sprouts, carrots, and scrambled eggs, dressed with sauce	Elementary and junior high school	107	23.0	78.0	10[e]	100	1×10^3	Frozen for 5 days
6	Three foods with sauce dressing	Boiled spinach, stewed carrots, deep-fried bean curd, and omelet, dressed with sauce	Nursery school	26	59.1	48.0	23[f]	40	9.2×10^2	Frozen for 7 days
7	Scallops boiled with cream sauce	Steam steeped scallops in sake broiled with egg, oil, and salt in oven	Hotel	30	79.0	32.5	2.0×10^4[g]	50	1.0×10^6	Refrigerated for 9 days
8	Cabbage, seachicken, and harusame, dressed with sauce	Cabbage, seachicken, and sticks of bean jelly, stir-fried with sauce	Elementary and junior high school	69	52.3	26.0	6.0×10^2[h]	100	6.0×10^4	Refrigerated for 2 days
9	Bavarois	Mixed hot milk with egg yolk, whipped cream, sugar, and gelatin, subsequently cooled	Junior college	100	81.3	22.5	3.0×10^3[i]	111	3.33×10^5	Refrigerated for 1 day

[a] Sample (10 g) diluted to 1:10, then five-tube MPN method with EEM, SBG, and DHL were used.
[b] Sample (10 g) diluted to 1:10, then five-tube MPN method with EEM, SS, and DHL used.
[c] Sample (10 g) diluted to 1:10, then five-tube MPN method with EEM, SBG, and DHL were used.
[d] Sample (0.1 ml of 10-g sample diluted 10 times) was plated on manitol lysine crystal violet brilliant green agar.
[e] Sample (10 g) diluted to 1:10, then five-tube MPN method with selenite broth and SS were used.
[f] Sample (5 g) diluted to 1:10, then three-tube MPN method using EEM, SS, and DHL.
[g] Diluted sample was directly plated.
[h] Sample (10 g) diluted to 1:10, then five-tube MPN method used.
[i] Sample (0.1 ml of 10-g sample diluted 10 times) was plated on DHL.

TABLE 2. *Median incubation period classified according to kind of causative cooking facilities*

Institution[a]	No. of incidents	Median incubation period (h)	SD	Groups showing significant associations[b]			
A	35	80.9	35.876	C	D	E	F
B	17	64.8	21.583	C	D	E	F
C	46	24.0	8.557	A	B		
D	50	26.7	10.887	A	B		
E	27	28.4	10.736	A	B		
F	10	26.6	12.186	A	B		
Total	185	—	—				

[a] A, elementary and junior high school lunches; B, nursery school lunches; and food prepared in: C, restaurants; D, take-out food shops; E, hotels; F, hospitals and welfare facilities.

[b] Differences in incubation period among six groups were examined by Scheffe's test after ANOVA ($P < 0.05$).

Attack rate for different cooking facilities. Mean attack rate by cooking facility was 46.1% for school lunches (33 incidents), 47.2% for nursery school lunches (17 incidents), 58.6% for restaurant foods (44 incidents), 51.2% for take-out foods (51 incidents), 49.6% for hotel foods (26 incidents), and 48.5% for hospital and welfare facility foods (10 incidents). However, no statistical correlation was observed between attack rates and type of facility.

Relationship between median incubation period and attack rate. As a result of the regression analysis of 50 food poisoning cases caused by school and nursery school lunches, a negative correlation was observed between the attack rate and the median incubation period ($y = -0.746x + 106.33$, $r = 0.491$, $P < 0.01$). Also as a result of the regression analysis of six cases limited to school and nursery school lunches of the nine cases shown in Table 1, a negative correlation was observed between the attack rate and the median incubation period ($y = -1.11x + 109.957$, $r = 0.856$, $P = 0.03$).

Elapsed time for different cooking facilities. In the Japanese school lunch system, foodstuffs are brought into the kitchens early in the morning of the day of use or in the afternoon of the day before and are used only for school lunches. These foodstuffs were cooked from 8:30 a.m. to noon in the kitchens and were eaten by 1 p.m. Although the nursery school lunches also were prepared by a similar process, the time for cooking in nursery schools is generally shorter than in other schools, the lunches being eaten between 10 a.m. and noon. Table 3 shows the average elapsed time for different cooking facilities. The average time between the start of the cooking process and consumption of food prepared by restaurants, take-out food shops, hotels, and hospital and welfare facilities ranged from 10.8 to 21.8 h. However, the required cooking times were short for school and nursery school lunches—4.8 and 2.9 h, respectively. The variability of data for restaurants, take-out food shops, hotels, and hospital and welfare facilities was greater than that for school and nursery school lunches. The average elapsed time of school lunches and nursery school

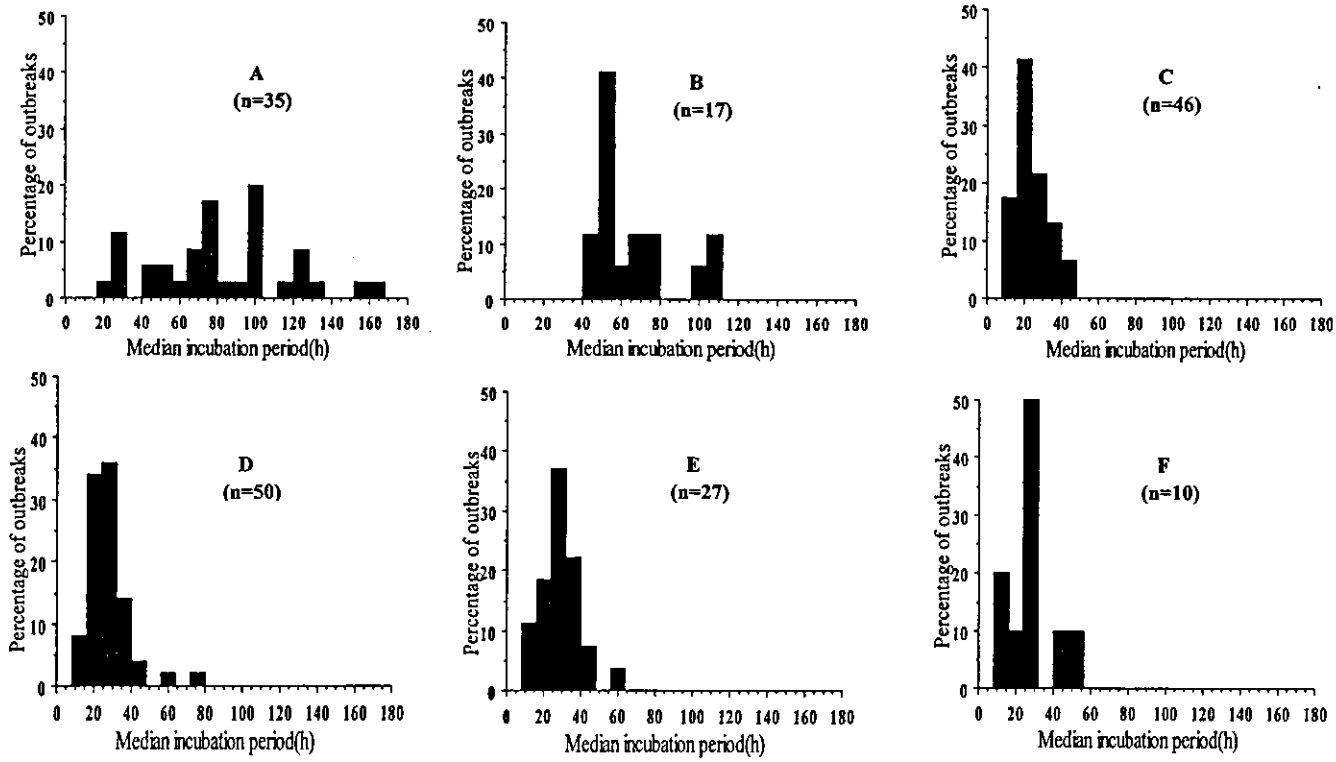

FIGURE 1. *Distribution of median incubation period of SE outbreaks (n = 185) classified according to kind of causative cooking facilities: (A) elementary and junior high school lunches, 35 outbreaks; (B) nursery school lunches, 17 outbreaks; food prepared in (C) restaurants, 46 outbreaks; (D) take-out food shops, 50 outbreaks; (E) hotels, 27 outbreaks; and (F) hospital and welfare facilities, 10 outbreaks.*

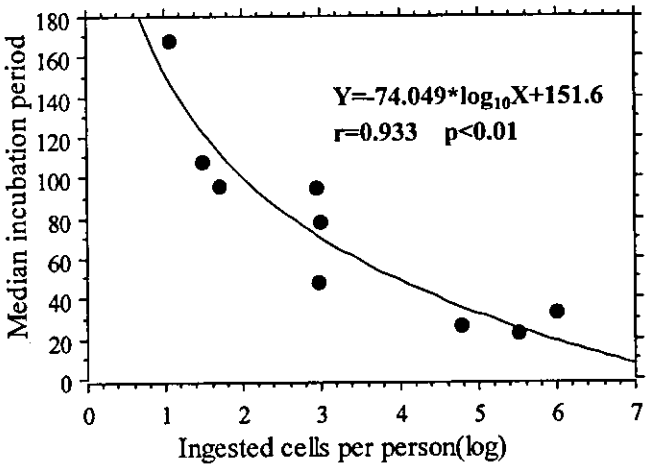

FIGURE 2. *Relationship between the median incubation period and the logarithmic bacterial dose ingested per person by nonlinear regression analysis.*

lunches was significantly shorter than that of food prepared by restaurants, take-out food shops, and hotels. However, no significant difference was observed in the average elapsed cooking time in restaurants, take-out food shops, hotels, and hospital and welfare facilities.

DISCUSSION

Gastroenteritis outbreaks caused by school and nursery school lunches have frequently been observed to have median incubation periods of more than 70 h, significantly longer than that for contaminated food prepared at other cooking facilities. Recognizing this, we analyzed the relationship between median incubation period and ingested dose per person in nine outbreaks of SE infection estimated with the use of SE concentrations in the causative foods (Table 1). According to the results of this survey, in outbreak 1 (168.0 h), with the longest median incubation period, the SE dose ingested per person was estimated at less than 12 cells. In addition, outbreaks 2 through 6 had median incubation periods longer than 48 h; the SE dose ingested per person in each outbreak was estimated at between 31 and about 10^3 cells.

Staff were advised to keep cooked foods as well as raw materials frozen at temperatures below −20°C until microbiological examination at large-scale cooking facilities could be performed under guidance provided by the Director General of the Environmental Sanitation Bureau of the Japanese Ministry of Health and Welfare. Freezing at −20°C for 90 days can leave the number of SE either unchanged or reduced by 1 log cycle *(5)*. Because the implicated foods were kept frozen below −20°C for 2 to 77 days or were refrigerated for 1 to 2 days until they were subjected to microbiological examination, it is reasonable to assume that the number of SE did not change significantly between the moment of consumption and the moment of enumeration and that the SE dose ingested per person estimated with the use of SE concentrations in the causative foods is credible. In a *Salmonella* Typhimurium infection study by Glynn and Palmer *(3)*, the incubation period was affected by the amount of contaminated food ingested; the

TABLE 3. *The time elapsed from the start of cooking to consumption of the contaminated food classified according to kind of causative cooking facilities*

Institution[a]	No. of incidents	Average elapsed time (h)[b]	SD	Groups showing significant associations[c]		
A	34	4.8	7.024	C	D	E
B	19	2.9	0.871	C	D	E
C	33	19.2	13.622	A	B	
D	44	17.4	10.621	A	B	
E	20	21.8	16.126	A	B	
F	10	10.8	2.835			
Total	160	—	—			

[a] A, elementary and junior high school lunches; B, nursery school lunches; and food prepared in: C, restaurants; D, take-out food shops; E, hotels; F, hospitals and welfare facilities.
[b] Elapsed time was calculated as the period from the start of cooking to the time of consumption of cooked food.
[c] Differences in elapsed time among six groups were examined by Scheffe's test after ANOVA ($P < 0.05$).

mean incubation period (20.7 h) for a group that ingested a small amount of contaminated foods was significantly longer than that (16.6 h) for a group that ingested more than twice the amount of contaminated foods. In addition to this report, similar reports show that the incubation period is affected by the amount of contaminated food ingested, although these data are within the range of the incubation period for common *Salmonella* infections *(7, 11)*. These results could be explained by the assumption that the incubation period of *Salmonella* infections is affected by the bacterial dose ingested. In addition, through our analysis here, we demonstrated that the median incubation period is prolonged to more than 48 h when the SE dose ingested per person is less than about 10^3 cells.

As a result of the analysis of 50 food poisoning cases caused by school and nursery school lunches, a negative correlation was observed between the attack rate and the median incubation period. Furthermore, the regression analysis of six cases limited to school and nursery school lunches for which bacterial concentrations could be determined also showed a negative correlation between attack rate and median incubation period. In short, the attack rates were low in SE food poisoning cases with long incubation periods. The above results also suggest that, in food poisoning cases caused by *Salmonella* in which bacteria doses are low, attack rates are low and incubation periods are long.

Because we observed that the long incubation period for SE infection was related to the characteristics of the cooking methods of school and nursery school lunches, we analyzed the data of each outbreak, focusing on the elapsed time between the start of the cooking process and the time of consumption. According to the results of this survey, the average elapsed time was 4.8 h for school lunches and 2.9 h for nursery school lunches. These periods were significantly shorter than those for food prepared in restaurants (19.2 h), take-out food shops (17.4 h), and hotels (21.8 h). A shorter cooking process and time period immediately be-

fore serving allows less time for the proliferation of bacteria; therefore, the number of ingested cells is probably very small.

The previous discussion leads to the assumption that the short elapsed time characteristic of school lunches and nursery school lunches affected SE outbreaks, resulting in prolonged incubation periods. Because it is possible that food ingredients or competitive bacteria have an effect on the survival and growth of *Salmonella*, it is necessary to perform more detailed investigations into the characteristics of the methods of cooking in causative facilities.

The attack rate in food poisoning cases caused by *Salmonella* is greatly affected by age of the infected person. Here, we noted that the average age of patients affected by contaminated food prepared in restaurants, take-out food shops, hotels, and hospital and welfare facilities was 37 to 55 years. On the other hand, many of the *Salmonella* infection victims at elementary and junior high schools and nursery schools were young—less than 10.6 years old. It is possible that the age of an individual also contributes to a long incubation period. We plan to study the relationship between age and incubation period further.

The attack rate between food poisoning cases in schools and nursery schools and cases in other categories of facilities were not different, despite a longer incubation period for the school and nursery school category, possibly because food poisoning remained latent at the other facilities because the attack rate is low for adults in food poisoning cases with long incubation periods. In future studies, we will proceed with an analysis of the effects of age and other factors. In this study, we gathered and analyzed only SE cases in order to eliminate, as much as possible, the effects of the difference in pathogenicity by *Salmonella* serotype on the infectious dose. However, differences in virulence by strain could be present. This possibility should be a subject of future research.

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Ribotyping and a Study of Transmission of *Staphylococcus aureus* Collected from Food Preparation Facilities

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ABSTRACT

Food poisoning from *Staphylococcus aureus* is sometimes caused by improper handling of food items in food preparation facilities. Prevention of contamination by employees is particularly important in facilities where a significant amount of food preparation is performed by hand. Some experiments have been performed to describe bacterial cross-contamination in the food preparation process, but there have been few studies of cross-contamination in actual food preparation facilities. Aiming to shed light on the transmission of *S. aureus* in food preparation facilities, this study collected samples of 66 strains of this bacterium from the fingers of food preparation staff, foodstuffs, prepared foods, cooking utensils, and cooking equipment and typed them with the ribotyping method. *S. aureus* from the same ribogroup was detected on the hands of a study participant, a faucet, knife, frying pan, and a salad, indicating that bacteria found on the hands of the study participant was transmitted to cooking utensils and prepared foods. Transmission (from a faucet to a frying pan handle) of bacteria by another person, a third party, was also detected.

In Japan, the number of food poisoning cases due to *Staphylococcus aureus* is trending lower because of factors such as improvements in personal hygiene management and improved facilities and equipment. However, large-scale incidents of food poisoning arising from contaminated processed milk occurred during the year 2000, affecting 14,780 people *(3)*, and *S. aureus* gained wide notoriety among the general public as a significant cause of food poisoning. Significant sources of food poisoning due to *S. aureus* include processed foods made of rice (such as Japanese onigiri) and other grains. Next in order of frequency are foods such as side dishes, which result from complex cooking processes, confectionery, and packaged meals *(2)*. In Japan, mechanization and automation of the preparation process have led to a decline in food poisoning cases attributable to onigiri contaminated with *S. aureus (15)*. However, a relatively large proportion of preparation work consisting of manual labor is required for cooked foods and confectionery. Therefore, the complete elimination of *S. aureus* from hands, utensils, and equipment involved in the manufacturing, processing, and sale of these foods is necessary. Most of these food products are manufactured and processed by relatively small facilities, and it is extremely important for purposes of preventing contamination that conditions with respect to *S. aureus* contamination and transmission in these facilities be clearly understood.

Model experiments have determined that transmission takes place among the hands of workers, cutting boards, faucets, the surfaces of foodstuffs *(6, 20, 21, 32)*, and fabrics *(24)*. Furthermore, pathogenic microbes transmitted through cross-contamination have been detected on raw materials, hands, and utensils in food preparation facilities. Therefore, it is necessary for people handling food in these places to understand and practice effective hygiene procedures *(8, 27)*. The purpose of this research is to demonstrate pathways of the transmission of *S. aureus* in a food preparation environment with involving personnel and, in particular, to shed light on the transmission of *S. aureus* from hands to cooking utensils and then to foods.

Recently, genotyping, an analytical method used in molecular biology, has been widely used in immunological and infection source studies. Its use in immunological studies of methicillin-resistant *S. aureus*, which has become a problem as a source of hospital-acquired infection, has been particularly prominent *(17)*. Genotyping can be performed through several methods, including ribotyping *(12, 22)*, pulsed-field gel electrophoresis (PFGE) *(12, 26, 28)*, amplified fragment length polymorphism (AFLP) *(7, 9, 31)*, and random amplified polymorphic DNA analysis *(19, 29)*. Hahm et al. *(10)* genotyped 54 strains of *Escherichia coli* using ribotyping, PFGE, AFLP, and repetitive extragenic palindromic PCR. These techniques were used for discriminating the O157:H7 isolate.

Ribotyping is one of the more convenient DNA fingerprinting analysis methods *(5, 30)*. Equipment that can automatically identify ribotypes in 8 h has simplified the identification of microbes and infection sources *(4, 12)*. However, little information is available concerning the ability of the ribotyping to characterize the movement and

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TABLE 1. *Sources and number of strains of 66 identified strains of* Staphylococcus aureus

Source	Contaminated sources/ sources tested	No. of strains
Study participants		
Hands (before food preparation activities)	4/26	25
Hands (during preparation activities)	3/26	15
Food		
Foodstuffs (chicken)	2/28	6
Prepared food (salad)	1/26	7
Utensils, facilities		
Frying pans	1/2	4
Faucets	1/2	5
Knives	2/26	3
Chopsticks	1/26	1
Total	15/162	66

transmission of a bacterial strain. The objective of this study was to reaffirm the possibility of *S. aureus* transfer among people and the others in food preparation environments. In this study, ribotypes were used to characterize the transmission and cross-contamination of *S. aureus* collected from the hands of workers, foodstuffs, prepared foods, utensils, and equipment.

MATERIALS AND METHODS

Collection of samples. Samples were collected from 10 places on kitchen countertops used by a total of 26 people. This kitchen is usually used as a cooking laboratory of Nagoya College of Nutrition. Samples were taken from the hands of workers both before and during their food preparation activities and from 28 types of foodstuffs, 26 prepared foods, 52 utensils, and 4 pieces of equipment.

The glove-juice method *(1)* was used to collect samples from the hands of workers. Before the collection of each sample, each of the 26 participants was asked to thoroughly rub their hands together. Samples were taken from the left and right hands; one hand was sampled before food preparation activities and the other during food preparation activities.

Samples, consisting of approximately 10 g of each test item, were also taken from foodstuffs and prepared foods. To prepare sample solutions for bacterial testing, sterilized saline solution (0.85% NaCl) was added to each sample, multiplying the original volume 10-fold, and the sample was homogenized in a stomacher (Masticator 400S, GSI Creos Co. Ltd., Tokyo, Japan). For faucets, frying pans, and other types of equipment and facilities, samples were obtained by using the wipe test. Knives and cooking chopsticks that were handled by study participants were inserted into sterile bags, and then bacterial samples were collected by using a massage method similar to the glove-juice method.

Isolation of bacteria. After serial dilutions, test solutions were incubated for 48 h at 35°C, using a commercially available egg yolk mannitol salt agar medium (Nissui Pharmaceuticals Co., Ltd., Tokyo, Japan), and colonies were identified by a yellow coloration surrounded by opaque egg yolk reaction. Each isolate from a colony was stored at −80°C until it was subjected to further analysis. Each strain was cultured overnight using tryptone soya broth (TSB) and then incubated at 35°C overnight using a TSB agar medium for identification.

Identification of isolates. Each isolated strain was identified with the Gram staining method (B&M Yamanaka method, Merck Ltd., Tokyo, Japan), coagulase test (rabbit plasma, Eiken Chemical Co., Ltd., Tokyo, Japan), separate coagulase-type test (Seiken immune serum for *S. aureus* coagulase typing, Denka Seiken Co., Ltd., Tokyo, Japan), and an identification test (APISTAPH, bioMérieux Japan Ltd., Tokyo, Japan).

Ribotyping. The RiboPrinter Microbial Characterization System (Qualicon, Wilmington, Del.) was used for ribotyping. Ribotyping is accomplished by using a restriction enzyme, *Eco*RI, to cut the DNA of an isolated strain into fragments. Then electrophoresis is used to separate DNA fragments containing rRNA genes, and then hybridization to labeled probes is performed. The banding pattern that results is then analyzed as the riboprint pattern and compared with a standard database.

Riboprint pattern analysis. The banding pattern of each isolated strain was retained as graphic data using the RiboPrinter Microbial Characterization System standard and then printed using the RiboPrinter System algorithm. Bacterial strains with the same banding pattern were given the same ribogroup name.

RESULTS

Identification of strains. Of 162 specimens, 15 yielded 66 different strains of *S. aureus* (Table 1). All 66 of the isolated strains were identified as *S. aureus* based on both gram-positive and coagulase-positive reactions. The 66 strains of *S. aureus* fell into the various coagulase types as follows: type I, 1 strain; type II, 3 strains; type III, 2 strains; type IV, 0 strains; type V, 34 strains; type VI, 0 strains; type VII, 1 strain; type VIII, 4 strains; and indeterminate, 21 strains (Table 2).

Riboprint pattern analysis. Riboprint pattern analysis resulted in the classification of the 66 strains of *S. aureus* into 39 ribogroups. Of these, 34 strains were from A to G groups, with each group comprising 2 to 10 strains. The other 32 strains were all given individual ribogroup names (Table 2). Comparisons by coagulase type and ribogroup show that some strains classified into different ribogroups may be of the same coagulase type. The seven ribogroups,

TABLE 2. *Origins, coagulase types, and ribogroups of the 66 isolated strains*

Isolate code	Origin	Coagulase type	Ribogroup	Group name[a]
K-03	Hand of study participant 2B[b]	Indeterminate	150-S-1	
K-04	(before food preparation activities)	Indeterminate	150-S-2	
K-05		Indeterminate	150-S-3	B
K-06		Indeterminate	150-S-4	
K-07	Hand of study participant 4C[b]	VII	132-S-7	
K-08	(before food preparation activities)	Indeterminate	150-S-6	
K-09		Indeterminate	150-S-7	
K-10	Hand of study participant 2A[b]	Indeterminate	150-S-8	
K-11	(before food preparation activities)	Indeterminate	151-S-1	
K-12		Indeterminate	151-S-2	
K-13		Indeterminate	151-S-3	
K-14		Indeterminate	151-S-4	
K-15		Indeterminate	151-S-5	
K-16		Indeterminate	151-S-6	
K-17		I	151-S-7	
K-18		Indeterminate	151-S-8	
K-19		Indeterminate	150-S-3	B
K-20		Indeterminate	152-S-2	
K-21		Indeterminate	152-S-3	
K-22	Hand of study participant 2A[b]	Indeterminate	150-S-3	B
K-23	(during food preparation activities	Indeterminate	152-S-5	
K-24	Hand of study participant 7B[b]	II	152-S-6	
K-25	(during food preparation activities)	II	152-S-7	
K-26	Knife (used by study participant 7A[b])	III	152-S-8	
K-27	Hand of study participant 7B[b] (during food preparation activities)	II	161-S-1	
K-29	Knife (used by participant 8B[b])	V	157-S-6	F
K-30		V	153-S-4	C
K-31	Hand of study participant 8B[b]	V	156-S-1	D
K-32	(before food preparation activities)	V	153-S-2	
K-33		V	156-S-1	D
K-34		V	156-S-1	D
K-35		V	153-S-4	C
K-36		V	132-S-2	A
K-37	Hand of study participant 8B[b]	V	132-S-2	A
K-38	(during food preparation activities)	V	156-S-1	D
K-39		V	156-S-1	D
K-40		V	157-S-7	G
K-41		V	157-S-7	G
K-42		V	157-S-6	F
K-43		V	157-S-6	F
K-44		V	153-S-4	C
K-45		V	153-S-4	C
K-46		V	155-S-3	
K-51	Chopsticks (used by participant 6C[b])	VIII	155-S-7	
K-53	Frying pan	V	156-S-1	D
K-54		V	156-S-1	D
K-55		V	156-S-1	D
K-56		V	156-S-1	D
K-57	Water faucet	V	156-S-1	D
K-58		III	156-S-6	
K-59		V	153-S-4	C
K-60		V	153-S-4	C
K-61		V	132-S-2	A
K-63	Foodstuff (chicken)	VIII	165-S-4	
K-65		VIII	157-S-2	
K-66		Indeterminate	157-S-3	E
K-68		Indeterminate	157-S-3	E
K-69		VIII	165-S-5	

TABLE 2. *Continued*

Isolate code	Origin	Coagulase type	Ribogroup	Group name[a]
K-71	Prepared food (salad prepared by participant 8B[b])	V	157-S-5	
K-72		V	157-S-6	F
K-73		V	157-S-7	G
K-74		V	157-S-7	G
K-75		V	153-S-4	C
K-76		V	153-S-4	C
K-77		V	165-S-3	
K-80	Foodstuff (chicken)	V	165-S-8	

[a] Group names were assigned when a ribogroup included two or more strains. Examples are as follows: 132-S-2, A; 150-S-3, B; 153-S-4, C; 156-S-1, D; 157-S-3, E; 157-S-6, F; and 157-S-7, G.
[b] Identifies study participant.

origins of the 34 strains, coagulase types, and riboprint patterns are shown in Table 3.

As summarized in Table 3, groups A, C, and D were isolated from the hand of participant 8B and a water faucet. Additionally, group D was isolated from a frying pan handle. In addition to the hand of participant 8B and the water faucet, group C was isolated from a knife and salad. Groups F and G were also isolated from the hand, cooked salad, and touched knife of participant 8B. These five groups are all of coagulase type V. Group B was isolated from the hands of two members of the same food preparation team, whereas group E was isolated from ground meat of chicken. The coagulase types of these two groups could not be determined since all of the immune serums for the various types failed to coagulate.

DISCUSSION

Hatakka et al. *(11)* collected the 153 hand samples from flight-catering staff, and *S. aureus* was isolated from 9% of samples. Irikura et al. *(16)* reported that 17.1% of 1,414 food handlers had *S. aureus* on their hands, and Sekiguchi et al. *(25)* isolated 93 strains (12.6%) from nostrils

TABLE 3. *The seven ribogroups, origins, coagulase types, and ribprint patterns*

Group Name	RiboGroup	Isolate code	Origin1	Origin2	Food preparation activities	coagulase type	RiboPrint pattern
A	132-S-2	K-36	Human	Hand of "8B"	prior	V	
		K-37			during	V	
		K-61	Faucet	Touched by "8B"	prior	V	
B	150-S-3	K-05	Human	Hand of "2B"	prior	Indeterminate	
		K-19		Hand of "2A"	prior	Indeterminate	
		K-22			during	Indeterminate	
C	153-S-4	K-30	Knife	Touched by "8B"	during	V	
		K-35	Human	Hand of "8B"	prior	V	
		K-44			during	V	
		K-45				V	
		K-59	Faucet	Touched by "8B"	prior	V	
		K-60				V	
		K-75	Salad	Cooked by "8B"	during	V	
		K-76				V	
D	156-S-1	K-31	Human	Hand of "8B"	prior	V	
		K-33				V	
		K-34				V	
		K-38			during	V	
		K-39				V	
		K-53	Frying pan	Touched by "anyone"	during	V	
		K-54				V	
		K-55				V	
		K-56				V	
		K-57	Faucet	Touched by "8B"	prior	V	
E	157-S-3	K-66	Food	Ground meat of	prior	Indeterminate	
		K-68		Chicken		Indeterminate	
F	157-S-6	K-29	Knife	Touched by "8B"	during	V	
		K-42	Human	Hand of "8B"	during	V	
		K-43				V	
		K-72	Salad	Cooked by "8B"	during	V	
G	157-S-7	K-40	Human	Hand of "8B"	during	V	
		K-41				V	
		K-73	Salad	Cooked by "8B"	during	V	
		K-74				V