

Shigella as a Foodborne Pathogen

ABSTRACT

Shigellosis is classically thought of as a waterborne disease; however, public health data suggest that foodborne outbreaks are a significant, if not the major cause of the disease in the United States. The role of *Shigella* as a foodborne pathogen is reviewed, including discussions of taxonomy, epidemiology, virulence factors, growth and survival in foods and model systems, and methods for detection/identification in food products.

Most food microbiologists consider the gastrointestinal disease caused by *Shigella* species as being mainly waterborne; however, evidence indicates that the foodborne route is quite important in terms of *Shigella*-induced infections. Black et al. (6) presented data which indicate that for the period 1961-1975, there were 72 outbreaks (10,648 cases) of foodborne shigellosis while there was only 38 outbreaks (5893 cases) for waterborne *Shigella* infections. For the period 1973-1982, there were 60 outbreaks (4519 cases) of foodborne shigellosis (17-23,64), but only 21 cases of waterborne shigellosis for the period 1971-1978 (24). From the public health data, it would appear that foodborne *Shigella* infections are more common than waterborne infections.

The host range of *Shigella* species is limited to higher primates including humans. The number of *Shigella* cells required to initiate an infection appears to be quite low—of the order of 10^1 to 10^4 cells/person (14). The organisms are transmitted through food and/or water contaminated by fecal matter from infected individuals with poor personal hygiene (58). Given the low infective dose, the consumer who eats food prepared by such individuals is at considerable risk of contracting shigellosis.

Mossel (71) stated that while the reported incidence of foodborne shigellosis is lower than that of salmonellosis, the problem of foodborne *Shigella* infections is probably greater than is realized. As the methodology for detecting *Shigella* species in foods improves, an evaluation of the true incidence of foodborne shigellosis can be made. We feel that foodborne shigellosis has been a neglected area of study and we wish to acquaint food microbiologists

with various aspects of the *Shigella* organism and with its behavior as a foodborne pathogen.

THE MICROORGANISM—TAXONOMY AND GENERAL CHARACTERISTICS

The genus *Shigella* is located in the family *Enterobacteriaceae*. Rowe and Gross (99) describe the genus as:

"Straight rods similar in morphology to other *Enterobacteriaceae*. Gram-negative. Nonmotile. Facultatively anaerobic, having both a respiratory and a fermentative type of metabolism. Catalase-positive (with exception of one species). Oxidase-negative. Chemoorganotrophic. Ferment sugars without gas production (a few exceptions produce gas). Do not use citrate or malonate as a sole carbon source. Do not grow in KCN or produce H_2S . Intestinal pathogens of man and other primates, causing bacillary dysentery. The mole% G + C of the DNA is 49-53."

In addition, *Shigella* species lack lysine decarboxylase, phenylalanine deaminase, and urease. They do not use gluconate as a sole source of carbon, do not liquefy gelatin, and are Voges-Proskauer negative but methyl red-positive.

Each of the four species of *Shigella*—*S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei*—contain serovars (*S. flexneri* also has subserovars) which are antigenically distinct (99). The species of *Shigella* may be differentiated by various chemical tests (Table 1.) Colicin and phage-typing have been found useful in studies of the epidemiology of *Shigella* outbreaks (99).

Brenner (10) has pointed out that *Shigella* and *Escherichia coli* are a single species on the basis of their DNA homology (70-100% related). The two genera can be difficult to separate on the basis of biochemical tests since there are gas-producing shigellae as well as lactose-negative, non-gas producing and nonmotile *E. coli* strains. Additionally, there are *E. coli* strains that produce a toxin similar to that produced by *Shigella* species and a similar disease is caused (72,109). Brenner (10) summed up the *Shigella-E. coli* problem by stating "Shigellae are actually metabolically inactive biotypes of *E. coli*."

TABLE 1. *Characteristics of Shigella species.*

Characteristics	Species of <i>Shigella</i>			
	<i>S. dysenteriae</i>	<i>S. flexneri</i>	<i>S. boydii</i>	<i>S. sonnei</i>
Serovars	1-10	1-6 with subserovars 1a, 1b; 2a, 2b; 3a, 3b, 3c; 4a, 4b	1-15	1
β -Galactosidase	(-) ^b ; serovar 1(+); other serovars may be (+)	(-); strains of serovar 2a (+)	(-); strains of serovar 9(+)	(+)
Growth of Na acetate	(-)	(-); strains of serovar 4 (+)	(-)	(-)
Ornithine decarboxylase	(-)	(-)	(-); strains of serovar 13 (+)	(+)
Indole	serovar 1 (-); serovar 2 (+); other serovars (\pm)	serovar 6 (-); other serovars (\pm)	(\pm)	(-)
Acid from glucose	(+)	(+)	(+)	(+)
Gas from glucose	(-)	(-); strains of serovar 6 (+)	(-); strains of serovar 13 and 14 (+)	(-)
Acid from lactose	(-)	(-); strains of serovar 2a (+)	(-); strains of serovar 9 (+)	(+) after several days
Acid from mannitol	(-)	(+); strains of serovars 4a and 6 (-)	(+)	(+)
Acid from sucrose	(-)	(-)	(-)	(+) after several days
Acid from dulcitol	(-); strains of serovar 5 (+)	(-); strains of serovar 6 (+)	(-); serovars 2, 3, 4, 6 and 10 (+)	(-)
Acid from raffinose	(-)	(\pm)	(-)	(+) after several days
Acid from xylose	(-)	(-)	(\pm)	(\pm)
Catalase	(+); serovar 1 (-)	(+)	(+)	(+)

^aTable modified from Rowe and Gross (99).

^b(-) = negative; (+) = positive; (\pm) = variable.

THE DISEASE AND ITS EPIDEMIOLOGY

The incubation period for shigellosis is 12-50 h after ingestion of the organism. Affected individuals demonstrate a gastrointestinal syndrome with diarrhea in most cases. Classical dysentery is marked by frequent evacuation of stools containing blood, mucus, and inflammatory cells. The individual also suffers abdominal cramps and tenesmus, ulceration of the intestinal mucosa, and fluid and electrolyte loss into the intestinal lumen. In a typical case, symptoms last for about 4 d, but severe cases may continue 10-14 d. During the acute stage, shigellae are found in large numbers in the feces and the organisms may be found in the feces for several weeks after symptoms have subsided. *S. dysenteriae* tends to cause the most severe illness; *S. sonnei* causes a mild disease, whereas *S. flexneri* and *S. boydii* infections can be either mild or severe. The mild type of infection (diarrhea) involves the proximal small intestine, whereas the more severe disease (dysentery) involves the colon. All four species penetrate the epithelial cells of the intestinal mucosa by attaching to the surface of the epithelial cell and are then engulfed by invagination of the epithelial cell membrane. The shigellae multiply within the cell and invade adjacent epithelial cells, culminating in ulcerative

lesions. Toxin released by the multiplying shigellae probably accounts for the tissue damage. Lesions found in the colon vary in severity from mild acute inflammation to diffuse ulcerative lesions (5, 41, 58, 86, 99a).

Models for studying shigellosis are few in number since humans and sub-human primates are the only known natural hosts. Data obtained from starved guinea pigs, ligated ileal loops and invasion of epithelial cells (cornea and tissue culture) (Table 2) correlate well with primate and human studies and can be used to provide insight into the infective processes of the *Shigella* organism and to demonstrate virulence in *Shigella* isolates.

Proper fluid replacement appears to be the best treatment for shigellosis (4). Antibiotic therapy may not always be necessary or desirable since resistance to antibiotics is widespread and increasing in the genus *Shigella*. The ampicillin resistance of shigellae isolated in England and Wales increased from 2% of the isolates in 1974 to 24% in 1978 (43). The number of isolates resistant to tetracycline increased from 15 to 51% during the same period. Increases in resistance to sulfonamide, streptomycin, and chloramphenicol also were noted (43). Gedebo and Tassew (40) isolated a number of shigellae from diarrheic patients and found resistance to one or more antimicrobial drugs in 85% of the isolates while 72% were

TABLE 2. Bioassays for invasive shigellosis^a.

Model	Effects note
Oral feeding of sub-human primates (Rhesus monkeys)	Similar clinical picture as humans with diarrhea, intestinal lesions with shigellae in intestinal epithelial cells
Oral feeding of starved, opiated guinea pigs	Ulcerative lesions in intestinal tract and death. Shigellae found in intestinal epithelial cells
Injection of shigellae into ligated ileal loops of rabbit	Fluid accumulation
Instillation of shigellae to eye of rabbits, guinea pigs or mice	Invasion of corneal epithelial cells with production of keratoconjunctivitis
Tissue culture cells (HeLa cells)	Invasion and multiplication of shigellae within epithelial cells with destruction of cell monolayer

^aData combined from references 38,41,62,73.

resistant to two or more drugs. In a recent report, Chugh et al. (27) examined clinical isolates of shigellae and found that resistance to one and two antimicrobial drugs were 3.9 and 12.4%, respectively; 77.8% of the shigellae isolates were resistant to three or more compounds and 58.0% were resistant to five or more drugs. In outbreaks of severe shigellosis when antibiotic treatment is deemed necessary, presence of multiple resistant strains makes shigellosis difficult to treat and eradicate.

Serious chronic consequences can result from repeated insults to the individual by bacterially-induced diarrhea (including those induced by *Shigella*). Diarrhea caused by certain enteric bacteria is believed to be an underlying cause of chronic rheumatoid diseases such as reactive arthritis and Reiter's syndrome (2,3,55).

Shigella infections are an important cause of diarrhea in developing countries (44). Children under 5 years of age are particularly at risk; in tropical developing countries, shigellosis accounts for at least 500,000 deaths/year in young children (44). Diarrhea caused by enterotoxigenic *E. coli* and *Shigella* retards the growth of children; the *E. coli* infections decreased weight gains whereas *Shigella* infections retarded height gains in children (1). *Shigella* infections also are considered to be the second most common cause of travelers' diarrhea (33).

Of all foodborne disease outbreaks in the U. S. caused by bacterial, chemical, parasitic, and viral agents for the years 1973-1982, the number of foodborne outbreaks due to *Shigella* species [an outbreak is confirmed by isolation of *Shigella* from implicated food or water and/or from the stools of infected individuals (23)] ranged from 1.5 to 6.3% and the number of cases ranged from 1.1 to 18.0% of the total (Table 3). Two deaths (one each in 1976 and 1979) were attributed to foodborne shigellosis during 1975-1982 (17-23,64); since there were 2919 cases of foodborne shigellosis during that period, the fatality rate was quite low. It is believed that only one in

TABLE 3. Confirmed foodborne disease outbreaks and cases due to *Shigella*, 1973-1982^a.

Year	Number		Number	
	of outbreaks	(% of total) ^b	of cases	(% of total)
1973	8	(6.3)	1388	(18.0)
1974	3	(1.5)	212	(2.3)
1975	3	(1.6)	413	(5.6)
1976	6	(4.5)	273	(7.6)
1977	5	(3.2)	67	(1.6)
1978	4	(2.6)	159	(3.2)
1979	7	(4.0)	356	(4.8)
1980	11	(5.0)	1184	(15.3)
1981	9	(3.6)	351	(4.1)
1982	4	(1.8)	116	(1.1)

^aFrom Annual Summaries of Foodborne Diseases (17-23,64).

^b% of total foodborne illness from bacterial, chemical, parasitic, and viral agents.

20 cases of shigellosis is reported (7); thus the number of actual cases of *Shigella*-induced disease must have been higher than the 2919 cases reported for 1975-1982.

During the period 1961-1975, of the 110 reported foodborne and waterborne *Shigella* outbreaks in the U. S., 68 were caused by *S. sonnei* and 34 were due to *S. flexneri*; the remainder were caused by unknown *Shigella* species (6). In the period 1975-1981, 18 of the 45 reported outbreaks were due to *S. sonnei*, 14 were due to *S. flexneri*, one was caused by *S. boydii* and two were due to unknown *Shigella* species (Table 4). Approximately 70% of the 93,516 *Shigella* isolates from humans that were reported to the Centers for Disease Control during 1974-1980 were *S. sonnei* (7). Since only 0.7% of the isolates were identified as *S. dysenteriae*, shigellosis induced by *S. dysenteriae* appears to be rather rare in the U.S. while *S. sonnei* causes most outbreaks. In 1984, 12,790 shigellae isolates from humans were reported to the Centers for Disease Control; 64.4% of the 12,179 identified shigellae isolates were *S. sonnei* strains (25a). *S. flexneri* comprised 30.9%, 3.2% were *S. boydii*, and 1.5% were *S. dysenteriae* (25a). The highest rate of shigellae isolations were obtained from children 1 to 4 years of age with the highest rate among 2-year-old children (7,26a). Outbreaks of foodborne shigellosis occur most frequently during the late spring to early fall (Table 5), but they do occur regularly during the year. Waterborne shigellosis also occurs with greatest frequency during the summer months (6), but as with foodborne shigellosis, cases occur throughout the year.

The chief contributing factor which led to foodborne *Shigella* outbreaks during the period 1961-1982 was poor personal hygiene of a food handler (i.e., failure to wash hands properly after defecating and subsequently handling food). Improper holding temperature of the contaminated foods was the second most important contributing factor (Table 6 and ref 6).

Most outbreaks were caused by food prepared and mis-handled in foodservice establishments (Table 4 and ref

TABLE 4. Foodborne disease outbreaks due to *Shigella*—vehicle, place where food was mishandled, and place where food was eaten, 1975-1981^a.

Year	<i>Shigella</i> species	Number of cases	Vehicle	Place where food was	
				Mishandled	Eaten
1975	<i>S. sonnei</i>	144	?	Food SE ^b	camp
	<i>S. sonnei</i>	150	?	Food SE	restaurant
1976	<i>S. flexneri</i> 2B	119	potato salad	Food SE	picnic
	<i>S. flexneri</i> 3a	46	?	Food SE	hotel
	<i>S. species unknown</i>	12	fruit compote	Food SE	sorority house
	<i>S. sonnei</i>	5	chopped liver	Food SE	restaurant
	<i>S. boydii</i>	176	spaghetti	Food SE	military mess hall
	<i>S. flexneri</i> 2	13	polynesian food	Food SE	youth center
	<i>S. sonnei</i>	21	tossed salad	Home	picnic
1977	<i>S. sonnei</i>	2	mexican food	Food SE	home
	<i>S. sonnei</i>	12	roast chicken, chili con carne, atole	Home	home
1978	<i>S. flexneri</i>	6	cream puff dessert	Food SE	airline
	<i>S. sonnei</i>	38	punch, potato salad	Home	school
	<i>S. flexneri</i> 2a	9	shrimp, raw clams	Food SE	roadside vendor
	<i>S. sonnei</i>	117	potato salad	Home	banquet hall
	<i>S. sonnei</i>	36	?	?	banquet hall
	<i>S. sonnei</i>	2	?	?	restaurant
	<i>S. sonnei</i>	4	chicken salad	?	home
1979	<i>S. flexneri</i>	26	shellfish	Food SE	camp
	<i>S. species unknown</i>	11	?	Food SE	restaurant
	<i>S. sonnei</i>	5	shellfish	Home	home
	<i>S. flexneri</i>	8	?	Home	home
	<i>S. sonnei</i>	13	mexican food	(probable ^c)	
	<i>S. sonnei</i>	280	fish salad	Food SE	restaurant
	<i>S. sonnei</i>	13	?	Home	cafeteria
1980	<i>S. flexneri</i>	15	meat (?)	Home	home
	<i>S. sonnei</i>	39	tuna	(probable)	
	<i>S. sonnei</i>	164	potato salad	?	?
	<i>S. flexneri</i> 4a	800	potato salad	Food SE	camp
				Food SE	picnic
				(probable)	
	<i>S. flexneri</i>	24	potato salad	?	?
	<i>S. sonnei</i>	3	?	Food SE	delicatessen
	<i>S. sonnei</i>	20	potato salad	?	?
	<i>S. sonnei</i>	47	?	?	?
	<i>S. sonnei</i>	38	?	Food SE	restaurant
	<i>S. sonnei</i>	8	fish	Food SE	restaurant
<i>S. flexneri</i>	26	?	Home	picnic	
1981	<i>S. flexneri</i>	7	?	(probable)	
	<i>S. sonnei</i>	20	potato salad	Food SE	restaurant
	<i>S. flexneri</i>	23	salad	?	?
	<i>S. sonnei</i>	119	?	Food SE	restaurant
	<i>S. sonnei</i>	76	?	?	?
				Food SE	school
	<i>S. flexneri</i>	32	chicken	(probable)	
				Home	church
				(probable)	
<i>S. sonnei</i>	14	?	Food SE	restaurant	
<i>S. sonnei</i>	42	potato salad	Food SE	school	
<i>S. sonnei</i>	18	?	Home	home	
			(probable)		

^aFrom Annual Summaries of Foodborne Diseases (17-23).

^bFood SE = Food Service Establishment.

^cThe Annual Summary did not make it completely clear just where the food was mishandled.

TABLE 5. Foodborne disease outbreaks caused by *Shigella* species—month of occurrences, 1961-1982.

Month	Number of outbreaks		Total
	1961-1975 ^a	1976-1982 ^b	
January	5	0	5
February	3	2	5
March	3	0	3
April	2	3	5
May	5	6	11
June	5	4	9
July	8	2	10
August	6	6	12
September	10	9	19
October	9	4	13
November	5	4	9
December	3	4	7

^aData from Black et al. (6).

^bData from Annual Summaries Foodborne Disease Outbreaks (18-23,64).

6), although foods prepared in homes were also identified in outbreaks. Many of the foods that caused problems were eaten away from home. Foods most often implicated in foodborne shigellosis were potato salads and salads with chicken or fish (Table 4 and ref. 6). This is probably due to salads being prepared from raw ingredients and/or previously cooked products without reheating. A variety of other foods were also implicated including meats and seafoods (Table 4 and ref. 6).

Details of some typical outbreaks of foodborne *Shigella* infections are presented below:

1. *S. sonnei* caused an outbreak at a county fair among individuals who ate at a smorgasbord. The implicated foods were potato, tuna, and egg salads, and cole slaw. The salads were prepared on the fairgrounds in large quantities 24 h in advance of serving. Of 400-500 individuals who ate the suspect meal, 248 cases of shigellosis were identified; the attack rate was 50-60%. Ill individuals complained of diarrhea (92%), fever (78%), and abdominal pain (76%). Fever and vomiting occurred in approximately 50% of the ill persons. The median incubation time before symptoms appeared was approximately 21 h.

All of the suspect foods were prepared, handled, and served separately but the salad dressing was common to all of the salads. It was postulated that an asymptomatic person was involved in preparation of the salad dressing and contaminated it with *S. sonnei*. Investigation demonstrated that many foods at the fair were improperly refrigerated, were kept at room temperature for long periods during preparation and serving, and there was no check made of the health of the food handlers. Proper training in large scale food preparation and handling should have prevented this outbreak (119.)

2. During field exercises at a military installation, 176 of 850 soldiers became ill with diarrhea; 53 were hospitalized. The disease was characterized by rapid onset with fever, abdominal cramps, and profuse diarrhea (bloody in some cases). Mean duration of the illness was 4 d with a range of 1 to 8 d; the mean incubation period was 50.5 h. Stool cultures indicated that the disease was caused by *S. boydii* 2 and the suspect food was spaghetti. Of 26 food handlers at the mess hall, 12 had diarrhea; one of the food handlers involved in the preparation of the spaghetti had diarrhea during preparation of the food and his stool was positive for *S. boydii* (he had spent the previous weekend in Mexico). The meat sauce and spaghetti were prepared several hours in advance but were said to have been heated before serving (18,p.55).
3. Two-hundred and eighty employees and visitors who had eaten at a hospital cafeteria complained of vomiting and/or diarrhea; 142 of the individuals had stools positive for *S. sonnei*. Eating food from the salad bar and/or eating tuna salad were significantly associated with the disease outbreak. One cafeteria employee had diarrhea on the first day of the outbreak; she had been previously exposed to a child with severe diarrhea. The employee had a stool positive for *S. sonnei*. This employee was responsible for all salad preparation and probably contaminated the tuna salad and other foods. (19, p. 31).
4. Six members of a family who had shared a common meal (rice, cooked fish with vegetables, lentil soup and fresh pineapple) were ill with vomiting, diarrhea, and fever. Three members of the family who did not eat the suspect meal remained well. The organism was identified as an unusual mannitol-positive *S. dysenteriae*. The incubation period was 14.5 h (range 12.0-16.5). The water supply which was used by several other families was not implicated; however, the actual food vehicle was not identified. (53).

TABLE 6. Foodborne disease outbreaks due to *Shigella*—contributing factors 1975-1982^a.

Item	1975	1976	1977	1978	1979	1980	1981	1982
Number of outbreaks	3	6	5	4	7	11	9	4
Number of outbreaks in which contributing factor reported	2	3	3	1	5	9	6	4
Contributing factors								
Poor personal hygiene	2	2	3	1	4	6	6	4
Contaminated equipment	1	—	—	—	—	—	1	—
Improper holding temperature	—	1	—	1	2	5	2	1
Inadequate cooking	—	2	—	—	—	—	—	—
Other	—	2	—	—	—	1	—	—

^aFrom Annual Summaries of Foodborne Disease (17-23,64).

5. *S. dysenteriae* 2 was responsible for dysentery in 107 individuals who ate raw vegetables from the salad bar in a military cafeteria. Patients had chills, fever, and abdominal cramps, with profuse watery or bloody diarrhea; nausea, vomiting, and dehydration were common. Duration of the illness ranged from 3 to 8 d. Gastrointestinal illness had been a common cause of absenteeism among food handlers at the cafeteria for the 3-week period before the outbreak. None of the food handlers, however, had positive stool cultures, and none had traveled to a foreign country recently (25).

It would appear that any food—generally of the type that is not heated after being prepared and of the type that is easily held at the wrong temperature—which is handled by a *Shigella* infected employee with poor personal hygiene can be a vehicle of foodborne shigellosis.

VIRULENCE IN SHIGELLA

Virulence in Shigella flexneri.

Virulent strains of *S. flexneri* (serotypes 1b, 2a, 4b, 5, and 6) contain a 140 Mdal plasmid. Avirulent strains either show large deletions in the 140 Mdal plasmid or lack it completely (101); the large plasmid also is probably involved with virulence in the other *S. flexneri* serotypes. *S. flexneri* loses the 140 Mdal plasmid at frequencies approaching one cell in 10^6 cells (116). Smooth colonial forms of *S. flexneri* which become avirulent upon losing the 140 Mdal plasmid are not altered in their group and type antigenic characteristics. Thus the 140 Mdal plasmid does not code for the LPS components in *S. flexneri* (101) in contrast to the behavior of the large plasmid (120 Mdal) in *S. sonnei* which does code for the specific LPS components (100). Transfer of the 140 Mdal plasmid from *S. flexneri* 2a into avirulent *S. flexneri* 1b rendered the latter virulent toward both tissue culture cells and guinea pig cornea (116). However, the antigenic expression was that of the recipient cell—1b, giving additional proof that the large plasmid does not code for antigenic determination. Using smooth virulent and avirulent strains of *S. flexneri* 2a, Okamura et al. (89) isolated rough mutants and tested them for their ability to invade HeLa cells and produce keratoconjunctivitis. Rough variants isolated from smooth virulent strains were able to penetrate HeLa cells but were not active against guinea pig cornea; rough strains from smooth avirulent *S. flexneri* were unable to invade either HeLa cells or guinea pig cornea. When specific O-antigen chromosomal genes from a smooth avirulent strain were introduced into virulent rough strains, the rough variants then became smooth and were able to invade both guinea pig cornea and HeLa cells (89). Thus production of the smooth colonial form depended on chromosomal genes and not plasmid genes in *S. flexneri*.

Enteroinvasive *E. coli* strains that produce keratoconjunctivitis and invade HeLa cells also harbor a 140 Mdal plasmid (48,103). The 140 Mdal plasmid from *S. flexneri* (mobilized by a transposon) rendered *E. coli* virulent

when inserted into avirulent enteroinvasive *E. coli* cells which had previously lost their 140 Mdal plasmid. However, the virulence plasmid (120 Mdal) from *S. sonnei* did not restore virulence to avirulent enteroinvasive *E. coli* (103). Hale et al. (47) and Boileau et al. (8) have shown that the virulence plasmids of *Shigella* species and enteroinvasive *E. coli* have a high degree of homology.

There is a close relationship between infectivity of enteroinvasive *E. coli* strains and aerobactin synthesis and transport. In a low-iron medium, *S. flexneri* produces aerobactin, a hydroxamate siderophore as well as a 76,000 dalton outer membrane protein which is assumed to be the receptor for ferri-aerobactin (42,95). The genes for aerobactin synthesis and transport are closely linked chromosomal genes in *S. flexneri* (63). These genes have considerable homology with the plasmid ColV aerobactin genes found in *E. coli*.

ColV plasmid-bearing strains of *E. coli* produce the siderophore aerobactin (9,115); the genes for aerobactin synthesis and transport of ferri-aerobactin are located on the ColV plasmid (120). Colicin production and aerobactin activity, while located on the same plasmid, are independent. Invasive strains of *E. coli* lacking the ColV plasmid or strains sustaining lesions in the plasmid genes involved in aerobactin synthesis or transport are not lethal when injected into mice (120). It is probable that aerobactin synthesis and transport is an important aspect of virulence in *S. flexneri*; however, studies to determine the role of aerobactin in virulence in *Shigella* species need to be done.

The expression of virulence in *S. flexneri* is also controlled by temperature. *S. flexneri* 2a grown at 37°C invaded Henle cells, produced keratoconjunctivitis, and absorbed congo red, whereas cells grown at 30°C or avirulent *S. flexneri* 2a grown at either 30 or 37°C lacked these characteristics (68). The plasmid profile of the virulent *S. flexneri* was the same at both temperatures; thus the lack of virulence at 30°C was not due to loss of the virulence plasmid. Virulent *S. flexneri* grown to 30°C and then transferred to 37°C were, after approximately 2 h, able to express virulence (68). Induction of virulence by a temperature upshift from 30 to 37°C required protein synthesis since chloramphenicol addition at the time of upshift prevented the return to virulence. It would appear that *Shigella* have no need to express virulence at 30°C but when they sense the host environment (37°C), the virulence genes are turned on. Temperature, therefore, acts as a trigger for expression of virulence genes.

Absorption of congo red from an agar medium containing the dye appears to correlate with virulence in *Shigella* species (68). Spontaneous mutants of virulent strains of *S. flexneri* 2a which have lost the ability to absorb congo red (Pcr^-) also lost the ability to invade Henle epithelial cells (67). The Pcr^- genotype was always associated with the loss of virulence. Maurelli et al. (67) found a close relationship between loss of the 140 Mdal plasmid, congo red binding and virulence in *S. flexneri*. These workers inserted a transposon near the congo red binding site on

the plasmid and then prepared a transducing phage lysate which was used to transduce avirulent Pcu⁻ strains. However, none of the transductants were virulent and they absorbed congo red at both 30 and 37°C. Thus these Pcr⁺ transductants did not behave like the parental Pcr⁺ which absorbed congo red only at 37°C and invaded tissue culture cells (67,68). The Pcr⁻ were always avirulent but the Pcr⁺ could be either virulent or avirulent.

Daskaleros and Payne (29) and Chambers et al. (26) demonstrated that the ability to bind congo red was encoded in the 140 Mdal plasmid of *S. flexneri* 1b. However, insertion of restriction endonuclease fragments containing the congo red binding activity into Pcr⁻ *S. flexneri* did not restore virulence even though the cells could bind congo red. The ability to bind congo red is a convenient marker to determine virulence in wild-type strains of *Shigella*, but it does not appear to have a real relationship to virulence.

Shigella entry into the intestinal mucosa is the key event in shigellosis. Bacterial metabolic activity is necessary for virulent *S. flexneri* 2a to initiate infection in Henle cells since non-viable virulent *S. flexneri* does not initiate infection (45). Further studies indicated that inhibitors such as cytochalasin B and dibutyryl cyclic adenosine monophosphate, which interfere with the phagocytic process, also inhibit Henle cell infection by *S. flexneri* 2a (46). Metabolic inhibitors such as iodoacetate or dinitrophenol inhibited phagocytosis and thus prevented bacterial infection of Henle cell monolayers. Hale and coworkers (46), using electron microscopy, found that shigellae were present in Henle cells within membrane-bound structures resembling phagosomes. These workers suggested that *S. flexneri* was taken into the epithelial cells via endocytosis and the phagocytic signal was provided by virulent but not by avirulent *Shigella* strains. Recent work by Sansonetti et al. (104) indicates that the induction of phagocytosis of *S. flexneri* 5 by HeLa cells depended on the presence of the 140 Mdal virulence plasmid.

S. flexneri 2a produces a toxin similar to *S. dysenteriae* 1 toxin. Keusch and Jacewicz (57) have shown that *S. dysenteriae* 1 toxin and *S. flexneri* toxin behave similarly on Sephadex columns and are similar in heat stability; the *S. flexneri* toxin also was neutralized by *S. dysenteriae* 1 antitoxin. O'Brien et al. (85), also demonstrated that *S. flexneri* 2a produced a toxin similar to *S. dysenteriae* toxin. The toxin of *S. flexneri* was lethal to mice, was active in rabbit ileal loops, and was cytotoxic to HeLa cells. Additionally, *S. flexneri* antitoxin neutralized the toxin produced by *S. dysenteriae* (78,85). Toxin production by *S. flexneri* 5 does not appear to be a prerequisite for intracellular growth in HeLa cells. The ability to reproduce rapidly within epithelial cells appears to be correlated not with the ability to produce toxin but rather depends on the presence of the 140 Mdal virulence plasmid (104). Thus *S. flexneri*, like *S. sonnei*, produces a Shiga-like (similar to *S. dysenteriae* 1) toxin which has not been studied extensively. In view of the importance

of *S. flexneri* as the causation of dysentery, the toxin should be studied in more detail.

Virulence in *Shigella sonnei*

Virulent *S. sonnei* strains display a smooth appearance on agar which is described as Form I. Form I cells easily dissociate into Form II cells which produce rough colonies and are avirulent, i.e., do not induce keratoconjunctivitis in guinea pig cornea (61). The loss of virulence is correlated with the loss of a 120 Mdal plasmid. The plasmid codes for synthesis of the specific O side chains of the lipopolysaccharide layer (LPS) which gives the smooth colonial morphology to Form I cells. Cells lose the plasmid at rates ranging from 1 to 50% depending on the strain of *S. sonnei* (100). Sansonetti et al. (100), using a transposon mobilizing system (the 120 Mdal plasmid is not self transmissible), introduced the Form I plasmid into Form II cells. The Form II cells then demonstrated virulence (keratoconjunctivitis) and also synthesized the specific O antigen.

Binns et al. (5) isolated rough mutants of *S. sonnei* which contained the 120 Mdal plasmid but failed to produce the specific O antigen. These rough mutants were able to invade HeLa cells but did not produce keratoconjunctivitis. Thus expression of the O antigen is necessary for positive action of guinea pig cornea.

Production of keratoconjunctivitis and absorption of congo red were found in *S. sonnei* grown at 37°C but not with cells grown at 30°C. The failure of the cells grown at 30°C to express virulence was not due to loss of the 120 Mdal plasmid since the plasmid profiles were similar for cells grown at 37 or 30°C (68).

Keusch and Jacewicz (57) found that a strain of *S. sonnei* produced a toxin similar to that of *S. dysenteriae* 1. The preparations from the two organisms behaved similarly on Sephadex chromatography and both exhibited similar heat stability. Antibody against *S. dysenteriae* 1 toxin also neutralized the activity of the *S. sonnei* toxin (57). Since *S. sonnei* is isolated frequently during *Shigella* outbreaks, more studies are needed concerning its toxin and the role of toxin in disease.

Virulence in *Shigella dysenteriae*

A small 6 Mdal plasmid encodes for synthesis of specific O-antigen and virulence in *S. dysenteriae* serotype 1 (117). Loss of the small plasmid results in reduced virulence. Using a DNA probe containing the active fragment of the 6 Mdal plasmid that allowed O-antigen synthesis, Watanabe et al. (118) demonstrated that the active fragment was present in other isolates of *S. dysenteriae* 1 but not in other serotypes of *S. dysenteriae* nor in other *Shigella* species. Virulent isolates of *S. dysenteriae* 1 or 2 harbor a 140 Mdal plasmid and were able to invade HeLa cells and guinea pig cornea (102). Virulent strains spontaneously gave rise to avirulent strains which either lacked the 140 Mdal plasmid or sustained deletions to that plasmid. The avirulent strains produced smooth colonial forms and expressed the antigenic specificity of the virulent parental type, indicating that the 6 Mdal plasmid

TABLE 7. *Biological activities found with purified Shiga toxin^a.*

Observation	Effect
Neurotoxicity	Paralysis and lethality in animals injected parenterally with toxin
Cytotoxicity	Death of cells in tissue culture
Enterotoxicity	Fluid accumulation in rabbit ileal loops
Neuronotoxicity	Cytotoxic effect of rat neurons
Protein synthesis inhibition	Prevention of protein synthesis in both mammalian and bacterial systems by in-activating 60S ribosomes with resultant cessation of peptide chain elongation

^aTable modified from Keusch et al. (59).

was still intact. The data obtained by Sansonetti et al. (102) and Watanabe et al. (117,118) indicate that for *S. dysenteriae* 1 both the 6 and 140 Mdal plasmids are necessary for virulence.

As with other *Shigella* strains, temperature is important in the expression of virulence by *S. dysenteriae*. Cells of *S. dysenteriae* grown at 30°C were not invasive toward Henle epithelial cells, did not produce keratoconjunctivitis, and failed to absorb dye from congo red agar plates (68). Cells grown at 37°C were invasive and took up congo red. The plasmid profile was the same regardless of the growth temperature. Growth of *S. dysenteriae* at 30°C did not lead to a permanent loss of virulence because when such cells were shifted up to 37°C and were allowed to incubate at the higher temperature for 2-3 h, the cells gained the ability to invade Henle cells (68).

The classical Shiga toxin is an exotoxin produced by *S. dysenteriae* 1. The precise role that the toxin plays in shigellosis is not completely understood. Several biological activities have been associated with the toxin (Table 7); all of these activities are believed to be caused by a single polypeptide. Recently, Keusch et al. (57) and Cantey (16) reviewed certain aspects of the production, purification, and characterization of Shiga toxin and the role that the toxin might play in shigellosis.

Under normal cultural conditions, Shiga toxin is produced during the exponential stage of growth and is secreted into the periplasmic space of the cell. During the stationary growth phase, significant amounts of toxin appear in the external medium (30,59). Shiga toxin has been isolated and purified from cell lysates and spent culture medium of *S. dysenteriae* 1 (12,80,91,93). The toxin consists of two polypeptide units: the A subunit (30,500 to 32,000 daltons) and the B subunit (5,000 to 6,500 daltons). The intact toxin consists of one A subunit com-

bined with 5-7 B subunits to give a molecular weight of 64,000 to 68,000 daltons (59,93). The A subunit can be dissociated into A₁ (28,000 to 29,000 daltons) and A₂ (3,000 daltons) subunits which are linked by a disulfide bridge. Shiga toxin is not immunologically related to cholera toxin or to the heat-labile toxin of enterotoxigenic *E. coli* (80).

Shiga toxin binds to a glycoprotein of the HeLa cell surface (60). It is probable, by analogy to other A-B subunit molecules, that the B subunit of Shiga toxin is involved in binding of the toxin to the surface receptors of the mammalian target cells (32,59,93). After binding, the toxin is then quickly transported from the cell surface to the cell interior via endocytic transport (34,54,59). Entry of Shiga toxin into tissue culture cells is temperature-dependent (92). Cell intoxication does not take place at 20°C but does at 25 or 37°C. Inhibition of protein synthesis appears to be the primary toxic effect of Shiga toxin in the host cell (11). All of the observed effects caused by Shiga toxin (Table 1) follow from that initial protein synthesis inhibiting step.

When intact HeLa cell monolayers were incubated with various amounts of Shiga toxin, there was inhibition of both protein and DNA synthesis but RNA synthesis was only slightly affected (11,98). The toxin did not cause cell membrane damage nor did it appear to interfere with uptake of metabolites. There was no depletion of the intracellular amino acid pool nor was there inhibition of energy metabolism. Shiga toxin also did not exhibit a cytolytic action. Brown et al. (11) indicated that inhibition of protein and DNA synthesis was the basis of the cytotoxic activity of Shiga toxin. Since inhibition of protein synthesis was induced by one toxin molecule per 1000 ribosomes, it would appear that toxin plays an important role in *Shigella* virulence (11).

Shiga toxin is synthesized by the bacterial cell as a zymogen which must be modified before toxic action against cell-free protein synthesis can be demonstrated. Treatment of toxin with trypsin followed by urea and dithiothreitol dramatically increased the toxic effect against cell-free protein synthesis in mammalian systems (13,98). During the course of the disease, cellular proteases would probably activate toxin after the microorganisms penetrated the epithelial cells.

Shiga toxin, in mammalian and microbial cell-free protein synthesizing systems, inhibited polyuridylic acid-directed synthesis of polyphenylalanine (13,90,111). The toxin does not inhibit aminoacylation of t-RNA (111) nor does it affect peptide bond formation (98) but appears to act directly on the ribosomes to inhibit peptidyl elongation.

Shiga toxin A₁ fragment inactivated the function of 60S ribosomal subunits in vitro by some unknown enzymatic action (98) but probably involved inhibition of elongation of the polypeptide chain. Inactivation of the ribosomes by toxin could be stopped by adding Shiga antitoxin but inactivated ribosomes could not be restored to activity (92). Obrig et al. (88) proposed that Shiga

toxin acted as a ribonuclease and thus inactivated ribosomes so that elongation of the peptide chain was prevented. It is obvious that more work is needed to define precisely the nature of Shiga toxin in relation to inhibition of protein synthesis.

S. sonnei and *S. flexneri* produce a shiga-like toxin (57,78,85). In addition, other *Enterobacteriaceae* produce shiga-like toxins. *Salmonella* strains (58,108), hemorrhagic *E. coli* strains (52,77,82,84,87,105,108), enteropathogenic *E. coli* strains which lack heat-labile or heat-stable enterotoxins and enteroinvasive capacity (28,79,81) and *Vibrio* strains (83,108) produce toxins which are neutralized by Shiga antitoxin. The shiga-like toxin was not immunologically related to cholera toxin or to heat labile toxin (LT) of *E. coli* (108).

Little is known about the genes involved in the production of Shiga toxin. Synthesis of shiga-like toxin in certain strains of hemorrhagic *E. coli* is encoded by bacteriophages (87); however, this situation is not known to occur in *S. dysenteriae* (5). Recently, Timmis et al. (112) have shown that the determinants for Shiga toxin production are closely linked to the arginine E gene cluster in *S. dysenteriae* 1. Thus it would appear that the genes for production of toxin are chromosomal rather than plasmid-located. Hopefully, further studies on the genetics and regulation of Shiga toxin biosynthesis will be forthcoming.

Virulence in Shigella boydii

Virulence in *S. boydii* has received little attention. Virulent isolates of *S. boydii* harbor a 140 Mdal plasmid and can invade HeLa cells and guinea pig cornea (102). Avirulent strains derived from virulent *S. boydii* produced smooth colonies and expressed the original antigenic specificity of the virulent parent; however, they lacked the 140 Mdal plasmid and did not invade epithelial cells. Obviously, the 140 Mdal plasmid does not code for O antigenic determinants.

Similar to *S. flexneri*, *S. boydii* synthesized aerobactin and utilized it to transport iron for growth (63). The genes for both transport and synthesis of aerobactin are closely linked on the bacterial chromosome; these chromosomal genes share homology with *E. coli* Co1V plasmid aerobactin genes (63). The possible relationship of aerobactin synthesis and transport to virulence has not been studied.

GROWTH AND SURVIVAL OF SHIGELLA SPECIES

It has been stated that *Shigella* species are fragile and are not particularly resistant to environmental stresses (122). However, a brief survey of the literature suggests that shigellae are *not* fragile and can survive for several days under a variety of conditions some of which may be present in foods.

The survival of *S. sonnei* on inanimate objects was studied by Spicer (107) and Nakamura (74). The mean survival time for 10 strains of *S. sonnei* stored at -20°C on glass, cotton, wood, metal, or paper was 30.4 d

(range 10-47). At 15°C storage, the mean survival time was 12.6 d (range 1-28) and at 37°C, it was 4.9 d (range 0-13). There was little survival at 45°C (74). Survival of *S. sonnei* was poorest on metal or glass at all temperatures except -20°C. Spicer (107), using cotton thread impregnated with *S. sonnei*, found that the organism did not survive well at temperatures above 15°C when the relative humidity was 40-60%.

Fehlhaber (35), using 21 strains each of *S. sonnei* and *S. flexneri*, studied the effect of NaCl, nitrite, pH, and temperature on growth and survival of the organisms. All of the strains of both *S. sonnei* and *S. flexneri* survived for at least 4 d in a nutrient medium containing 6% NaCl but only one *S. sonnei* and two *S. flexneri* strains could survive 4 d in 10% NaCl. Neither organism survived in 15% NaCl (35). Nakamura et al. (76) found that *S. sonnei* could survive in NaCl solutions ranging from 0 to 25% for at least 9 d if the temperature was 25°C or less. *S. flexneri* appeared to be more sensitive to nitrite than *S. sonnei*; at pH 5.5, growth of *S. flexneri* was inhibited by 450 ppm as compared to 700 ppm nitrite necessary to inhibit *S. sonnei* (35).

All of the strains of *S. flexneri* and *S. sonnei* survived for 4 h in a nutrient medium poised at pH 4.0 or 4.5, but they only survived for 30 min at pH 3.5 (35). When Hentges (49) added organic acids (formic and acetic acids) to a synthetic medium and poised the pH at values ranging from 7.0 to 5.5, he found that growth of *S. flexneri* at 37°C was prevented at pH values equal to or less than 6.0; however, in medium lacking the organic acids, there was only slight inhibition at pH 5.5. In his studies, Fehlhaber (35) incubated the shigellae cultures at *Zimmertemperatur* (room temperature) which can be assumed to be 25-26°C.

Fehlhaber (35) found that *S. sonnei* strains grew at temperatures ranging from 7 to 46°C; none grew at 49°C. The *S. flexneri* strains had growth temperatures ranging from 10 to 44°C with none of the strains growing at 48°C. At 63°C, 18 of the *S. sonnei* strains and 20 of the *S. flexneri* survived for 1 min; very few of the strains survived 63°C for 5 min (35).

Taylor and Nakamura (110) studied survival of *S. sonnei* and *S. flexneri* 2a in certain foods. At temperatures equal to or less than 25°C, *S. sonnei* and *S. flexneri* survived more than 50 d in flour, milk, egg, shrimp, oysters, and clams. Survival in acidic products like orange juice, tomato juice, and carbonated soft drinks was much less—in the range of 5 to 10 d. Using strains of *Lactobacillus acidophilus* to ferment milk, Wilson and Tanner (121) showed that *S. dysenteriae* and *S. flexneri* survived 15 to 32 d when the pathogen and starter culture were added together (the milk was incubated at 37°C until coagulation, then it was placed at 8°C). When the shigellae were added to refrigerated fermented milk, the survival was 7 to 14 d. The pH of the milk products was 4.0 to 4.2 with titratable acidities ranging from 0.85 to 1.1%. Shigellae added to milk acidified directly with lactic acid survived only 1 d. (121).

ISOLATION AND DETECTION OF *SHIGELLA* SPECIES

There appear to be no data concerning the incidence of *Shigella* in raw food materials or finished food products. This lack of information is probably in great part due to the paucity of distinguishing biochemical characteristics in *Shigella* as well as due to the difficulty in separating *Shigella* from certain *E. coli* strains.

One of the difficulties with the selective agars and enrichment broths recommended for isolation of *Shigella* is that they may contain bile salts or deoxycholate which are inhibitory to injured cells (113). Presence of injured cells due to sublethal stress is a very real possibility in processed foods, particularly if the food has been under-processed (106). One of the consequences of injury is that injured cells can not repair themselves and produce colonies in the presence of toxic agents which are added to bacteriological media to confer selectivity (106).

Tollison and Johnson (113) showed that heat-injured *S. flexneri* cells do not repair themselves and form colonies on tryptic phyton glucose agar (TPGA) containing deoxycholate or bile salts even though colony formation by the injured cells was observed on TPGA alone. Since *Shigella* can cause disease when present in small numbers, use of selective agars containing bile salts or deoxycholate may lead to failure to detect small numbers of injured cells which could repair themselves in a food product (when the temperature is too high or there is other mishandling) and eventually lead to disease.

Tollison and Johnson (113) recommended plating dilutions of food samples suspected of containing injured shigellae onto a non-selective agar such as TPGA followed by incubation at 35°C to allow repair of injured cells. Then the non-selective agar was overlaid with TPGA containing deoxycholate or bile salts. They found that 8 h of cell repair on non-selective agar was optimum for heat-injured *S. flexneri*.

Nakamura and Dawson (75), studying *S. sonnei* injured by freezing and thawing, found that colony formation by injured cells was poor on a synthetic agar but excellent on a rich complex agar. Addition of meat extract, peptone, or casamino acids to the synthetic medium improved its ability to support colony formation by freeze-thaw injured *S. sonnei*. The metabolic lesion produced by freezing and thawing could be repaired only when proteinaceous nutrients were supplied. The data obtained by Nakamura and Dawson (75) indicate that injured *Shigella* species probably will have more demanding nutritional requirements and non-selective media used for repair of injured cells should reflect that increased nutritional requirement.

Other workers who have studied repair of injured bacterial cells have noted that addition of catalase or sodium pyruvate to selective agars increased colony formation by injured *S. aureus*, *Salmonella typhimurium*, *S. senftenberg*, *Pseudomonas fluorescens*, and *E. coli* on those selective agars (66,97). Flowers et al. (37) and Buckner et al. (15) presented evidence which indicated

that heat-injured *S. aureus* was more sensitive to H₂O₂ than non-injured cells and that peroxide prevented repair of the injured cells on selective agar. The addition of catalase or pyruvate (a non-enzymatic H₂O₂ decomposer) to selective agar led to decomposition of toxic peroxide and permitted repair and colony formation by the injured cells. It would be of interest to determine if addition of pyruvate (or catalase) to *Shigella* selective agars would allow increased isolation of shigellae from food products. For a discussion of injury and repair of injured microorganisms see Smith and Palumbo (106) and Ray (96).

Wilson and Tanner (121) recommended use of MacConkey agar (MAC) for isolation of *S. dysenteriae* and *S. flexneri* from milk products. SS agar was recommended as a second choice for isolation of *Shigella*. Morris et al. (70) compared MAC, xylose-lysine-deoxycholate (XLD) and SS agars for isolation of *Shigella* from fecal specimens. Direct plating of fecal specimens onto XLD agar was superior to the other agars for isolation of *S. sonnei* while both SS and XLD agars were superior to MAC for isolation of *S. flexneri*. Buffered glycerol saline solution was recommended as a transport medium when it was necessary to transport fecal samples to the laboratory. However, Morris and coworkers (70) found that direct plating of fecal samples in the field (i.e., patient's bedside) resulted in higher yields of shigellae than when the samples were transported to the laboratory for plating. Use of both XLD and SS agars for each sample also resulted in higher yields of *Shigella* species.

To isolate *Shigella* from foods, Twedt (114) recommended adding food samples to gram-negative broth and selenite cystine broth. After overnight incubation, MAC, XLD, deoxycholate citrate (DCC), and Levine's eosin-methylene blue (EMB) selective agars were streaked from the enrichment broths. *Shigella* species appear on XLD agar as rose-colored colonies surrounded by a rosy halo when viewed by transmitted light. On MAC, DCC, and EMB selective agars, *Shigella* colonies are translucent, colorless, and lactose-negative. At least two suspect colonies are picked from each plate and streaked on the slant and stabbed into the butt of triple sugar iron agar (TSI) slants. Tubes that show a red streak and yellow butt with no gas or H₂S were subjected to biochemical and serological tests for *Shigella* (114).

Morris (69), when isolating *Shigella* from foods, recommended adding food samples to gram-negative broth and adjusting the pH to 6.0-7.0. Following overnight incubation, two of the following selective agars were streaked from the enrichment broth: tergitol 7, MAC, XLD, SS, Hektoen enteric, or DCC agars. He particularly recommended use of MAC and XLD agars. Suspect colonies were inoculated into TSI slants. Further testing for *Shigella* species is similar to that of Twedt (114).

Various members of the family *Enterobacteriaceae* antagonize growth of *Shigella* species. *S. flexneri* did not compete well with strains of *Klebsiella*, *Proteus*, or *E. coli* in vitro (39,50,51). This antagonistic behavior of enteric microorganisms against shigellae must be taken into

consideration when isolations are made from food and fecal samples.

Pal and his associates (94) developed a rapid (24 h) method for detection of virulent *Shigella* species by use of an enzyme-linked immunosorbent assay (ELISA) based on recognition of the virulence marker antigen (VMA), which is common to the four *Shigella* species and to enteroinvasive *E. coli* strains. The ELISA correlated with the ability of the organism to produce keratoconjunctivitis and with the presence of the large virulence plasmid. Avirulent strains of enteroinvasive *E. coli* and shigellae did not give a positive ELISA. It should be possible to use the VMA ELISA on *Shigella* isolated from food products. An ELISA has been developed for detection of *Shigella* toxin in stools of patients (31). The assay was quite sensitive (12 pg) and specific for *Shigella* toxin.

CONCLUSIONS AND RECOMMENDATIONS

A survey of the literature indicates that *Shigella*-induced disease, at least in the U.S., is primarily a foodborne illness. Food microbiologists should be aware that *Shigella* species can cause a particularly severe form of food poisoning. They should actively search for organisms of that genus when food poisoning is suspected, particularly if large numbers of the common food poisoning genera (staphylococci, enterotoxigenic *E. coli*, *Salmonella*, or *Clostridium perfringens*) are not present.

Shigella species do not appear to be especially susceptible to environmental factors and appear to be able to survive for long periods in foods of many types and under a variety of conditions. It should be recognized that shigellae are at least as hardy as other enteric food poisoning bacteria. Since only a few organisms can initiate disease in susceptible individuals, *Shigella* species can undergo a more extensive 'die-off' than some of the other food poisoning bacteria and still be a danger when present in foods stored at the wrong temperatures.

Further studies are needed to determine the precise role of toxin in virulence of *Shigella* species. The available evidence using purified toxin suggests that toxin is important in vivo, but its effects have not been completely elucidated especially for toxin elaborated by the organism after it has invaded the epithelial cell. The genetics of toxin production and its regulation is poorly understood and more studies are needed to map the exact location of the genes involved in toxin biosynthesis. The mechanism(s) involved in regulation of *Shigella* toxin biosynthesis are, at the present time, unknown.

The classical techniques and media used for isolation, detection, and enumeration of *Shigella* species are neither very specific nor very sensitive. Progress in the study of shigellae as foodborne pathogens can not advance very far nor very fast under these conditions. The genus is not very active biochemically and the close relationship of *Shigella* species to *E. coli* makes it difficult to develop specific isolation techniques. It may be possible to detect and enumerate shigellae by using DNA colony hybridiza-

tion techniques (36); however, the close relationship to *E. coli* may preclude this methodology for absolute specificity. In fact, Boileau et al. (8) developed a DNA hybridization technique based on a probe containing a fragment of the large virulence plasmid from *S. flexneri* 5. The probe could be used to detect virulent strains of all four *Shigella* species as well as virulent strains of enteroinvasive *E. coli*.

Since injured cells are always a possibility in processed foods, the presence of injured shigellae must always be considered. Incorporation of a H₂O₂ scavenger such as pyruvate to the selective media should facilitate recovery of injured organisms.

Shigellosis appears to be a uniquely human disease transmitted by humans with poor personal hygiene and who handle or prepare foods. People who lack the elementary knowledge of sanitation and food preparation need to be identified and given training in sanitary habits and how to prepare foods in a safe manner. While education would appear to be one of the best control measures in prevention of shigellosis, Marth (65) points out that most foodhandlers are young and inexperienced and stay on the job less than a year; thus finding and educating these foodhandlers while they are actively working is difficult. Greater awareness on the part of microbiologists in the food industry and regulatory agencies will accomplish much in the control of foodborne shigellosis.

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