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Salmonella enterica subspecies *diarizonae* Bacteremia in an Infant with Enteritis
-A Case Report-

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The septicemia caused by the Arizona group organism is rare and usually observed in adults with underlying diseases. In Korea, Salmonella infection is common, but a report of Arizona infection is unknown. We isolated S. enterica subsp. diarizonae from blood of a 6-month-old infant. The serovar was determined as 28:z_{10}:-, a rare one in America. The isolate was susceptible to ampicillin, chloramphenicol, cotrimoxazole and others. The patient rapidly recovered with ampicillin and gentamicin therapy. Clinical laboratories should consider that the infection exists in Korea and should attempt to isolate and identify Arizona organism in certain patients.

Key Words: *S. enterica* subsp. *diarizonae*, Arizona, bacteremia

The Arizona group organism is now classified in the species of *Salmonella* because of its close relationship to *Salmonella* in biochemical, serological and genetic characteristics (Le Minor, 1984; Ewing, 1986). As the nomenclatural status of *Salmonella* has not been settled yet, the organism is still called by various names. *Salmonella enterica* subsp. *arizonae* and subsp. *diarizonae* are the names presently used by Ewing (1986).

Arizona group organisms differ from other salmonellae in that they are mostly isolated from reptiles such as snakes and turtles. Human infection is rare. Most of the patients are adults with various underlying dieasesd (Johnson *et al.*, 1976). The sources of isolations are mostly from feces and rarely from other specimens, such as, blood, urine, respiratory or wound (Weiss *et al.*, 1986).

Isolation of Arizona group organism was not previously known, in Korea. We isolated *S. enterica* subsp. *diarizonae* from the blood of a patient with fever, vomiting and diarrhea. The patient was a 6-month-old boy with no underlying disease. We report the clinical features of the patient and characteristics of the isolate to stress the serious nature of the infection in some cases and the difficulty in isolating the Arizona group organism from stool specimens.

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CASE REPORT

A 6-month-old male infant (Unit No. 2017030), living in Seoul, was transfered to Severance Hospital, Seoul, from a local clinic on June 2, 1989. He had had 4 days of fever and 4-5 vomitings daily with 7-8 watery diarrheas.

During physical examination on admission, he appeared acutely ill, but, except for increased bowel sounds, no abnormal findings were noted. He was a moderately developed boy weighing 8.3 kg with no previous history of illness or genetic abnormalities. He had received vaccinations of BCG, DPT, poliomyelitis and hepatitis B.

His chest X-ray was normal. Hematologic findings were: WBC count 12,200/μl with 47% segmented neutrophils, 49% lymphocytes and 4% monocytes, hemoglobin 122 g/l, hematocrit 37% and platelet count 522,000/μl. Serum electrolytes were Na 134 mmol/l, K 3.9 mmol/l, Cl 103 mmol/l, CO_2 10 mmol /l. Urine output was decreased, but a urinalysis was normal. Parasites including amoeba were not found in the feces. A stool culture did not yield *Salmonella, Shigella, Yersinia,* enteropathogenic *Escherichia coli* (EPEC), *Vibrio* and *Campylobacter*. From a blood cul-

ture, growth of gram-negative bacilli was detected (isolate No. 89-6-5241) and it was identified as *S. enterica* subsp. *diarizonae*. After 3 days of antimicrobial therapy of ampicillin and gentamicin, the fever was resolved, diarrhea stopped and urine output increased. On the 6th hospital day, the patient was discharged with complete recovery.

To isolate enteric pathogens from feces, MacConkey agar, SS agar, TCBS agar, selenite broth (Difco) and Butzler's Campylobacter selective agar (Oxoid) were inoculated. Campylobacter agar was incubated in a microaerophilic condition at 42°C for 48 hours and the other media were incubated at 35°C in a room air incubator. Selenite broth culture was subcultrued onto SS agar. Triple sugar iron (TSI) agar tubes were inoculated with colorless colonies on MacConkey and SS agar to detect *Salmonella* and *Shigella*. To detect EPEC, antisera (Difco) were used for slide agglutination test.

For blood culture, two 1-3 ml of blood samples were taken and inoculated into 30 ml vials of tryptic soy broth (TSB) and Brewer thioglycollate medium (Difco). Daily macroscopic observations and CO_2 analysis with BACTEC 660 (Johnston Laboratories, Towson, Md) were done to detect any growth of bacteria.

For the identification of blood culture isolate, conventional biochemical tests (Kelly et al., 1985) and API 20E (API Systems, S.A., Montalieu Vercieu, France) and Vitek GNI (Vitek Systems, Hazelwood, Mo.) were used. To determine the serovar, both O and H antigens were tested. Antimicrobial susceptibility was tested by the disk diffusion method (NCCLS, 1988).

RESULTS

A stool specimen was cultured, but no *Salmonella, Shigella, Yersinia,* EPEC, *Vibrio* nor *Campylobacter* were isolated. In one of two blood cultures, growth of gram-negative bacilli was detected in a TSB vial after 3 days incubation. On the MacConkey agar plate subcultured with the TSB, pink colonies were formed after overnight incubation.

With the conventional minimal tests the isolate showed biochemical reactions of *Salmonella,* i.e., TSI reactions of alkaline slant, acid butt and production of gas and H_2S, positive tests of motility, ornithine decarboxylase, lysine decarboxylase and Simmons citrate and negative test of indole prodution. Antimicrobial susceptibility pattern was also that of *Salmonella,* i.e., it was susceptible to ampicillin, cephalothin, cefamandole, cefoperazone, cefotaxime, ceftazidime, chloramphenicol, tetracycline, amikacin, gentamicin, tobramycin and cotrimoxazole.

The isolate, however, did not show agglutination with *Salmonella* polyvalent O antiserum containing antibodies against factors 1-16, 19, 22-25 and 34. Identifications with the API 20E and Vitek GNI card were *Salmonella arizonae*. Additional biochemical tests showed positive gelatin hydrolysis and acid production from lactose and galacturonate (Table 1). Acid production from lactose was detected after overnight incubation with both cystine tryptic agar base and purple broth base.

A further serological test showed that the antigenic formura of the isolate was $28{:}z_{10}{:}-$. Based on the results of the biochemical and serological tests, the isolate was identified as *S. enterica* subsp. *diarizonae*.

DISCUSSION

Arizona group organism was first isolated from reptiles in America by Caldwell and Ryerson in 1939. This organism has been called by various names such as *Salmonella arizonae. Paracolobacterium arizonae, Arizona arizonae, Arizona hinshawii* (Ewing, 1986). As the nomenclature of *Salmonella* has not yet been agreed upon, the organism is still called by various names. According to Le Minor (1984) Arizona group are classified as subgenus III of genus *Salmonella*. Ewing (1986) used the name, subsp. 3a, *S. enterica* subsp. *arizonae,* and subsp. 3b, *S. enterica* subsp. *diarizonae*.

Arizona group organisms are mostly isolated from lizards, snakes, turtles and turkeys. The organism is rarely isolated from man (Weiss *et al.,* 1986). In Korea, nontyphoidal *Salmonella* infections were more prevalent than typhoid fever during the Korean war (Chun, 1964; Chun, 1975; Chung. 1986) and since the late 1980s (Unpublished data at Severance Hospital), but Arizona infection was not found in literature. Although the present case documents the existence of Arizona infection in Korea, its prevalence is difficult to speculate, because the routine procedure of stool culture fails to detect lactose-positive Arizona group (Weiss *et al.,* 1986).

Besides gastroenteritis, Arizona group produces various infections such as septicemia, pneumonia, empyema, otitis media, brain abscess, meningitis, osteomyelitis, septic arthritis, hepatic abscess and others (Johnson *et al,*. 1976; McIntyre *et al.,* 1981; Petru and Richman, 1981). It was thought septicemia may accompany the gastroenteritis (Guckian *et al.,* 1967),

Table 1. Biochemical characteristics of *S. enterica* subsp. *diarizonae* isolate

Test or substrate	*S. enterica* subsp. *diarizonae**	Isolate No. 89-6-5241
H_2S (TSI agar)	+**	+
Urease	−	−
Indole	−	−
Methyl red	+	+
Voges-Proskauer	−	−
Simmons citrate	+	+
KCN	−	−
Motility	+	+
Gelatin	(+) or −	+
Lysine decarboxylase	+	+
Arginine dihydrolase	(+) or −	−
Ornithine decarboxylase	+	+
Phenylalanine deaminase	−	−
Gas from glucose	+	+
Acid from glucose	+	+
lactose	d	+
sucrose	−	+
mannitol	+	−
dulcitol	−	+
salicin	−	−
adonitol	−	−
inositol	−	−
sorbitol	+	−
arabinose	+	+
raffinose	−	+
rhamnose	+	−
maltose	+	+
xylose	+	+
trehalose	+	+
cellobiose	+	+
glycerol	− or +	+
erythritol	−	−
galacturonate	+	+
Malonate	+	+
Lipase	−	−
DNase	−	−
Esculin	−	−
Oxidase	−	−
Nitrate to nitrite	+	+
ONPG	+	+

*Adapted from Ewing, 1986.

**+, positive; −, negative; (+) delayed positive; d, +, (+) or −.

and certain Arizona serotypes are more prone to cause septicemia than others (Weiss *et al.*, 1986).

Weiss *et al.* (1986) reported that among the arizonae isolated during 1967-1976, 68% were from stool specimens, 9% from blood and 22% from other specimens such as urine, respiratory, wound, abscess and synovial fluid. Although the stool specimen was watery, we failed to isolate any pathogenic bacteria. Based on the fact that the Arizona isolate from blood culture showed pink colonies on MacConkey agar after overnight incubation and that we did not select pink colonies for the detection of enteric pathogen, it could be assumed that we missed the Arizona organism.

Most of the patients with Arizona infections were adults with various underlying diseases including HIV infected individuals (Noskin and Clarke, 1990). Among the 12 patients reviewed by Johnson *et al.* (1976) only two patients were 2 years old and one 21-year-old man did not have any underlying disease. It was noteworthy that our patient was a male infant just 6 months old without any underlying disease.

Egg powder, egg nog, ice cream and custards were most frequently incriminated in multiple case outbreaks of Arizona infection (Guckian *et al.*, 1967) and unpasteurized milk was the source in another patient (Johnson *et al.*, 1976). Rattlesnake meat capsules, a popular folk remedy in certain regions of the world, were reported to be heavily contaminated with arizonae and were the source in some sporadic infections (McIntyre *et al.*, 1981; Marzouk *et al.*, 1983; Riley *et al.*, 1988; Noskin and Clarke, 1990). Our patient's usual diet included both milk and soft food. Before the infection, he had completed a trip with his parents outside the city. It is possible that he had taken contaminated food during the trip, but, as is in most food borne diseases, it was impossible to speculate on the source of infection. Eating uncooked meat of various animals, a recently increased trend, or contaminated animal products may be a risk factor of Arizona infection in Korea, too.

Antimicrobial therapy should depend on the susceptibility pattern of the isolate, but, as with other *Salmonella* infections (Sperber and Schleupner, 1987), ampicillin, chloramphenicol and cotrimoxazole were considered effective for the treatment of Arizona infection (Johnson *et al.*, 1976; Petru and Richman, 1981; Riley *et al.*, 1988). Increased isolation of ampicillin- and chloramphenicol-resistant *Salmonlla*, especially *S. typhimurium*, was reported in Korea (Chong *et al.*, 1987), but the Arizona isolate was susceptible to all of the antimincrobial agents tested, and the patient made rapid rocovery after ampicillin and gentamicin treatment.

It is known that isolation of Arizona organism from the stool is difficult because lactose-nonfermenting colonies only are selected for further testing (Weiss *et al.*, 1986). Identifying the Arizona group may not

be simple either. At first the blood culture isolate was suspected to be a rare serotype of *Salmonella*, but it did not agglutinate with the polyvalent *Salmonella* antiserum, despite the fact that the minimal biochemical screening test reactions were those of *Salmonella*. However, using API 20E and Vitek GNI systems the organism was identified as Arizona group. Further conventional tests showed the isolate to have characteristics of Arizona group, i.e., dulcitol negative, β-galactosidase and lactose positive. The isolate was identified as *S. enterica* subsp. *diarizonae* based on the positive test of galacturonate and rapid acid production from lactose after overnight incubation, but the isolate did not show diphasic H antigen.

It is known that there are over 400 serotypes of Arizona organism. Weiss *et al.* (1986) reported that 374 strains of Arizona group isolated in 1967–1976 in America belonged to 71 serovars. Among the isolates 339 were *S. enterica* subsp. *arizonae* and 232 were *S. enterica* subsp. *diarizonae*. The most prevalent serovars were 18:z_4,z_{23}:– and 61:1,v:1,5. The serovar of our isolate, 28:z_{10}:–, was not found among the isolate list. This may indicate that different serovars of Arizona are distributed in Korea.

It is concluded from the study that Arizona infection exists in Korea and that the prevalent serovar may differ from those in America. In the bacteriological study of certain gastroenteritis patients who had eaten uncooked or contaminated animal products, the laboratory should also attempt to detect Arizona group. We need studies in Korea to expand our knowledge on this organism.

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