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Distinctive Biochemical Features of *Salmonella dublin* Isolated in California

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We examined 34 strains of *Salmonella dublin* that were isolated in California between 1978 and 1982. All were of a characteristic biotype; they did not grow on Simmons citrate or acetate and did not ferment arabinose. Their apparent inability to use citrate as the only carbon source was due to a nutritional requirement for nicotinic acid. Because *S. dublin* strains are of a characteristic biotype, are host adapted to bovines, and are unusually virulent for humans, we suggest that *S. dublin* be considered a separate species of the genus *Salmonella*. It is important that clinical laboratories recognize and differentiate this organism from less pathogenic salmonellae so that they can alert clinicians to the presence of this invasive microorganism.

Salmonella enteritidis subsp. *dublin* (*S. dublin*) is host adapted to cattle and rarely infects other animals (15). In cattle it causes either severe diarrhea in calves or a typhoid-like illness that often results in a chronic carrier state (7). In the last few years in California and neighboring states there has been an increase in the number of humans infected with *S. dublin* by ingesting raw milk (4). In humans *S. dublin* can cause diarrhea but more often produces sepsis and metastatic abscesses (16; J. Fierer, West. J. Med., in press); that is, *S. dublin* infections more closely resemble those caused by *S. cholerae-suis* than those caused by other serotypes of *S. enteritidis* (13). It was of interest to us, therefore, to find that the biochemical reactions of *S. dublin* were not typical of other serotypes of *S. enteritidis* and that it was possible to make a provisional identification of *S. dublin* before serotyping was done.

We examined 34 isolates of *S. dublin*. A total of 7 were from the Veterans Administration Medical Center, San Diego, Calif., 17 were from the California State Department of Health, Berkeley, and 10 were from the San Diego County Health Department, San Diego, Calif. All were identified as *S. dublin* by the California Department of Health Microbial Diseases Laboratory. All strains isolated in San Diego County were from human infections. Of the strains provided by the California State Department of Health, one was from a calf, four were from samples of raw milk, and the remainder were from human infections. All were isolated between 1978 and 1982.

The biochemical reactions of all the strains were determined with conventional media by the methods of Edwards and Ewing (6). Tubes were incubated for 72 h before they were designated as negative. The minimal medium developed by Neidhardt et al. (10) was used to determine the growth requirements of *S. dublin*. All the strains were inoculated onto solidified minimal medium with a replica plating device (Cathra International, Minneapolis, Minn.). Glucose was replaced with equimolar concentrations of the other carbohydrates that were tested.

The biotypes of all the *S. dublin* strains were determined by a modification of the method of Walton (14). We added 0.15% sodium deoxycholate to phenol red agar (BBL Microbiology Systems, Cockeysville, Md.) containing 1.0% (wt/vol) carbohydrate.

Antibiotic susceptibilities were determined by disk diffusion testing by the method of Barry et al. (1).

All isolates were of the same biotype (Table 1). None of the strains tested grew on Simmons citrate or acetate agar, and none fermented arabinose. In all other respects their reactions were typical of *S. enteritidis*. Because the citrate and acetate media are minimal media and because *S. dublin* is known to be a nicotinic acid auxotroph (2), we tested the effect of nicotinic acid on the ability of *S. dublin* to utilize citrate, glucose, acetate, and arabinose as sole carbon sources. None of the isolates grew without nicotinic acid (50 μg/ml) on minimal medium with any single carbon source. In the presence of nicotinic acid, all isolates utilized glucose and citrate but not acetate or arabinose. We then determined the biotype of each isolate based on

TABLE 1. Comparison of biochemical reactions of *S. dublin* and other group D salmonellae

Test	Reaction[a] of:		
	S. dublin[b]	*S. enteritidis*[c]	*S. typhi*[c]
H_2S (Kligler iron agar)	+ (100)	+ (98)	+[w] (94)
Citrate (Simmons)[d]	− (0)	+ (99)	− (0)
Gas	+ (100)	+ (98)	− (0)
Ornithine decarboxylase	+ (100)	+ (99)	− (0)
Arabinose	− (0)	+ (99)	− (6)
Rhamnose	+ (100)	+ (95)	− (0)
Acetate[d]	− (0)	+ (95)	− (0)
Vi antigen	− (3)	− (0)	+

[a] +, Positive reaction; w, weak reaction; −, negative reaction. The numbers in parentheses indicate the percentages with positive reactions.

[b] A total of 34 strains were tested.

[c] See reference 6.

[d] Media were inoculated from a saline suspension without carryover of nutrients. The inoculated media were incubated at 35°C and examined daily for 3 days. Negative reactions were determined after 72 h of incubation.

carbohydrate fermentations, as described by Walton (14). All the strains were biotype D (arabinose negative and rhamnose, trehalose, and glycerol positive).

Of the isolates, 16 were susceptible to all antibiotics tested. Only 1 isolate was resistant to ampicillin, carbenicillin, chloramphenicol, sulfamethoxazole, and tetracycline, 5 were resistant only to ampicillin and carbenicillin, and 15 were resistant to ampicillin, carbenicillin, and tetracycline. A high rate of antibiotic resistance in *S. dublin* isolates has also been noted in Europe and probably reflects the selective pressure of antibiotics in livestock feed (5).

We examined the biochemical features of 34 strains of *S. dublin* isolated in California between 1978 and 1982 and found that all the strains were of the same biotype. They were unable to utilize either citrate or acetate and did not ferment arabinose. In the presence of supplemental nicotinic acid, all strains grew on citrate medium but still did not utilize acetate. Thus, they were *nic* auxotrophs and also were unable to metabolize acetate because of some other defect in the anaplerotic pathway (9). Among group D salmonellae, this biochemical pattern is so distinctive that *S. dublin* can be provisionally identified if conventional media are used. We could not identify *S. dublin* with the API 20E system (Analytab Products, Plainview, N.Y.) because the citrate and arabinose reactions were variable and often gave false-negative results with other serotypes of *S. enteritidis* that we tested.

The biochemical characteristics of *S. dublin* are of practical importance and also may shed some light on the biology of this organism. Most salmonellae are capable of infecting many hosts, but a few, such as *S. typhi* and *S. dublin*, are very host adapted (11). The basis for host adaptation is largely unknown but may sometimes have a metabolic basis. For instance, *S. pullorum* is auxotrophic for cysteine, an amino acid that is found in abundance in chickens because it is utilized to synthesize feather proteins (8). *S. typhi* is a tryptophan auxotroph (12) but it is not known whether this relates to its ecology. We have confirmed that *S. dublin* is a nicotinic acid auxotroph (2). It is interesting that bovines can synthesize nicotinic acid and do not require niacin in their diet (3). It is possible, therefore, that *S. dublin* utilizes the host's niacin, thereby ensuring adequate nutrition even in an animal that may have an inadequate diet because of infection.

As we and others have noted, *S. dublin* often causes a systemic disease manifested as sepsis and metastatic infections in humans (16; Fierer, in press). Because *S. dublin* is so virulent and is also likely to be multidrug resistant, prompt identification by clinical laboratories is very important. A specific microbiological identification of *S. dublin* will alert physicians to search for sites of deep infection.

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