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High Incidence of Hemolysin Production by *Enterococcus (Streptococcus) faecalis* Strains Associated with Human Parenteral Infections

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Hemolysin production, clumping (pheromone) response, transferability of the hemolytic trait, and drug resistance were examined in 97 clinical isolates of *Enterococcus (Streptococcus) faecalis*. The isolates were derived from various sources (i.e., urine, pus, vagina, sputum, bile, and blood), and approximately 60% were found to be hemolytic. About 85% of the hemolytic strains exhibited a clumping response, compared with about 49% of the nonhemolytic strains. Over 50% of the hemolytic strains carried transferable hemolysin determinants, and in no case were drug resistance genes linked. The hemolytic strains exhibited multiple drug resistance more frequently than did the nonhemolytic strains. In contrast to the high frequency of hemolysin producers among parenteral isolates, strains derived from fecal specimens of healthy individuals exhibited a low (17%) incidence of hemolysin production.

Hemolysin production is common among gram-positive and gram-negative bacteria; however, in most cases its role in virulence is not clear. There have been reports that urinary tract infections caused by *Escherichia coli* are more likely to involve hemolytic strains (7, 8, 20). *Enterococcus (Streptococcus) faecalis*, a member of the normal intestinal flora, is frequently the causative agent of urinary tract infections and can be involved in other disease states such as endocarditis. *E. faecalis* subsp. *zymogenes* is distinguished from other *E. faecalis* strains by its production of a cytotoxin able to lyse human, rabbit, and horse erythrocytes. Strains producing this beta-hemolysin also produce a bacteriocin, and it is believed that the hemolysin and the bacteriocin are mediated by the same genetic determinant (1–3, 14). In several beta-hemolytic *E. faecalis* isolates examined to date, the hemolysin-bacteriocin trait has been associated with a conjugative plasmid which transfers in broth matings at a frequency of 10^{-3} to 10^{-1} within a few hours (3). This plasmid confers a mating response to small peptide sex pheromones excreted by potential recipient cells (9, 10). This mating signal induces the synthesis of a proteinaceous adhesin which coats the donor cell surface (25). The adhesin is referred to as aggregation substance and is believed to facilitate the formation of mating aggregates upon random collision between the nonmotile donors and recipients. Once a copy of plasmid DNA has been acquired by the recipient, the production of the related pheromone ceases; however, pheromones specific for donors harboring different classes of plasmids continue to be excreted (4, 6, 10, 16). Donor cells induced by exposure to culture filtrates of recipients undergo self-clumping; for this reason, the sex pheromone has been referred to as clumping-inducing agent. Aggregation substance is presumed to adhere to a binding substance which is located on the surface of both donor and recipient cells (4a).

Plasmid pAD1 (56.7 kilobases) in *E. faecalis* confers hemolysin-bacteriocin expression as well as a conjugative mating response to the sex pheromone designated cAD1 (5, 6, 15, 23). We reported recently that the hemolysin-bacteriocin determinant of pAD1 significantly enhanced virulence in intraperitoneal infections in mice (17). The role of *E. faecalis* hemolysin in human infections has been obscure; to our knowledge, no epidemiological data showing a correlation of the hemolytic trait with a specific type of human infection have been reported. In this communication, we report that a high proportion of *E. faecalis* clinical isolates in Japan are hemolytic, and the hemolytic traits are frequently encoded by a conjugative plasmid.

MATERIALS AND METHODS

Bacteria, media, and reagents. *E. faecalis* FA2-2 (rifampin resistant [Rif^r], fusidic acid resistant [Fus^r]) (5), JH2SS (streptomycin resistant [Str^r], spectinomycin resistant [Spc^r]) (22), OG1RF1 (Rif^r Fus^r) (10), and OG1-10 (Str^r) (9) were used as recipient strains. A total of 106 strains of group D streptococci were obtained from clinical sources. Of the 106 strains, 39 were received at Gunma University Hospital of Maebashi, Japan, between January and April 1983, and 67 were received at Isesaki City Hospital, Isesaki, Japan, between May 1983 and March 1984. The clinical isolates were from foci containing group D streptococci at a density of at least 10^5/g (net weight) of material. Unless otherwise indicated, the media used throughout this study were Oxoid nutrient broth 2 (Oxoid, Basingstoke, Hants, England) supplemented with glucose (0.2%), and Tris hydrochloride (0.1 M; pH 7.7) (N2GT broth) and Todd-Hewitt agar plates (Difco Laboratories, Detroit, Mich.). Antibiotic concentrations used in selective plates were as follows (in micrograms per milliliter): erythromycin, 25; streptomycin, 500; spectinomycin, 500; kanamycin, 500; gentamicin, 200; chloramphenicol, 25; tetracycline, 3; rifampin, 25; and fusidic acid, 25. Hemolysin detection was on Todd-Hewitt agar containing 4% human (Gunma University Hospital), horse, or rabbit blood (Toyo Serum Co., Tokyo, Japan). Bile esculin azide agar plates (Difco) were used in primary isolation media for group D streptococci.

Identification procedures for clinical isolates. The isolation

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TABLE 1. Presence of *E. faecalis* and *E. faecium* in clinical isolates containing group D streptococci and incidence of hemolysin production by isolates of *E. faecalis*

Source	No. of specimens			No. of hemolytic strains of *E. faecalis* (%)
	Total	With *E. faecium*	With *E. faecalis*	
Urine	52	2	50	25 (50)
Pus	23	3	20	14 (70)
Vagina	12		12	6 (50)
Sputum	7		7	6 (85)
Bile	5	3	2	2
Blood	2	1	1	1
Unknown	5		5	4
Total (%)	106 (100)	9 (8.5)	97 (91.5)	58 (60)

procedures used by the two hospitals were the same. One loopful (0.001 g) of material (specimen) was spread on bile esculin azide agar (Difco), and the plate was incubated overnight at 37°C. After the incubation, colonies were examined for color, size, and shape. Colonies were picked, purified, and Gram stained, and the species of group D streptococcus was identified immunologically by the Phadebact Strep D test (Pharmacia, Uppsula, Sweden) and then by the API 20 Strep system (Analytab Products, Plainview, N.Y.).

Identification procedures for fecal group D streptococci from healthy individuals. Group D streptococci were isolated from stool specimens of 100 healthy students at the Gunma University Medical School. One loopful (0.001 g) of each sample was spread on bile esculin azide agar (Difco); 10^1 to 10^4 colonies appeared on plates after overnight incubation at 37°C. *E. faecalis* colonies were black, large, and rough compared with those of *Enterococcus* (*Streptococcus*) *faecium* and *Streptococcus avium*, which were gray, small, and smooth. All of the colonies on each plate were uniform in appearance, except when both *E. faecalis* and *E. faecium* grew. Between 5 and 50 colonies were picked randomly from each plate and purified, and the morphology of these colonies was rechecked; the species of group D streptococcus was identified by the Phadebact Strep D test (Pharmacia) and by the API 20 Strep system (Analytab).

Determination of hydrolysis of gelatin. The medium for determining hydrolysis of gelatin was prepared by adding 30 g of gelatin to 1 liter of Todd-Hewitt agar (Difco). Bacteria were inoculated from colonies on the gelatin agar plate with a sterile toothpick, and the plates were incubated overnight (18 h) at 37°C. Hydrolysis of gelatin was determined by screening for the appearance of a turbid halo around the colony after the plates were cooled for 5 h at 4°C.

Drug resistance of strains. Overnight cultures of the strains in antibiotic medium 3 (Difco) broth were diluted 100 times with fresh antibiotic medium 3 broth. One loopful of each dilution was plated on antibiotic medium 3 plates, with one drug per plate, and the plates were incubated for 18 h at 37°C.

Mating procedures. Broth matings were performed as previously described (11, 18) with a donor/recipient ratio of 1:10. Overnight cultures of 0.05 ml of donor and 0.5 ml of recipient were added to 4.5 ml of fresh broth, and the mixtures were incubated at 37°C with gentle agitation for 4 h and then vortexed. Portions of the mixed culture were then plated on solid media with appropriate selective antibiotics. Colonies were counted after 48 h of incubation at 37°C. Filter matings were carried out as described previously (13) with Todd-Hewitt agar plates containing 4% human blood and with an initial ratio of 1 donor per 10 recipients. For the transfer of hemolysin properties, the mating mixtures were diluted 10^{-1}, 10^{-2}, and 10^{-4} times with fresh N2GT broth. A 0.1-ml sample of each dilution was plated on selective Todd-Hewitt agar plates containing 4% human blood and an appropriate drug for counterselection of the donor strain. After overnight incubation of the plates at 37°C, the colonies of recipients producing a hemolytic zone were counted as hemolytic transconjugants.

Clumping assay. Detection of aggregation (clumping) was as previously described (9, 10). Pheromone corresponded to a culture filtrate of the plasmid-free strain JH2-2. Generally, 0.5 ml of culture filtrate from late logarithmically growing cells was mixed with 0.5 ml of fresh N2GT broth and 20 μl of overnight cultured cells to be tested for ability to respond. The mixtures were cultured for 4 h at 37°C with shaking and were examined for clumping.

RESULTS

High frequency of *E. faecalis* among clinical isolates of Lancefield group D streptococci. Among 106 clinical isolates of group D streptococci, about 90% were *E. faecalis*; the others were *E. faecium* (Table 1). Among 100 healthy medical students at Gunma University, 23 had *E. faecalis* in stool specimens, whereas 84 harbored *E. faecium*. (More

TABLE 2. Pheromone response and drug resistance of Hly^+ and Hly^- strains of *E. faecalis*

Response and resistance patterns[a]	Isolation frequency[b] of strain		P[c]
	Hly^+ (n = 58) (%)	Hly^- (n = 39) (%)	
Positive clumping	49 (84.5)	19 (48.7)	<0.001
Drug susceptible	6 (10.3)	18 (46.2)	<0.001
Tc	12 (22.4)	8 (25.6)	
Sm	1		
Em		2	
Tc Em	3 (12.1)	3 (10.3)	
Tc Sm	1	1	
Tc Cm	2		
Tc Km	1		
Tc Cm Sm	1 (12.1)		
Tc Km Gm	1		
Tc Km Sm	1		
Tc Em Cm	1		
Tc Em Sm	2		
Tc Km Gm	1		
Tc Em Km		2 (5.1)	
Em Cm Km Gm	5 (13.8)		
Tc Em Km Gm	1		
Tc Em Sm Km	2	1 (7.7)	
Tc Em Cm Km		2	
Tc Em Cm Sm Km	2 (29.3)	2 (5.1)	
Tc Em Sm Km Gm	4		
Tc Em Cm Km Gm	11		

[a] The aggregate with exposure to pheromone was performed as indicated in Materials and Methods. Tc, Tetracycline resistance; Sm, streptomycin resistance; Em, erythromycin resistance; Cm, chloramphenicol resistance; Km, kanamycin resistance; Gm, gentamicin resistance.

[b] Hly^+, Hemolytic; Hly^-, nonhemolytic.

[c] Significance was determined by using the Fisher exact test for 2-by-2 contingency tables.

TABLE 3. Conjugative transfer of hemolysin production of *E. faecalis*[a]

Phenotype of donor strain	No. of Hem$^+$ transconjugants/ donor cell	Phenotype of transconjugant (%)[b]	No. of donor strains
Hly	10^{-2}–10^{-3}	Hly	3
Hly Tc	10^{-3}	Hly	8
Hly Tc Em	10^{-2}	Hly (10), Hly Em (90) (a)	2
Hly Tc Em	10^{-6}	Hly Em (b)	1
Hly Tc Cm	10^{-3}–10^{-4}	Hly (10), Hly Cm (90) (c)	2
Hly Tc Cm Sm	10^{-3}	Hly	1
Hly Tc Km Gm	10^{-3}	Hly (50), Hly Km Gm (50)(d)	1
Hly Tc Em Sm Km	10^{-3}	Hly	1
Hly Em Cm Km Gm	10^{-4}	Hly	1
Hly Tc Em Cm Sm Km	10^{-3}	Hly	1
Hly Tc Em Cm Km Gm	10^{-3}	Hly (10–20), Hly Em Cm Km Gm (90–80) (e)	8
Hly Tc Em Cm Km Gm	10^{-3}	Hly (10), Hly Em Cm Km Gm (80), Hly Em Cm (10) (f)	1
Hly Tc Em Cm Km Gm	10^{-3}	Hly (10), Hly Km Gm (90) (g)	2

[a] Overnight cultures of donors of Hly$^+$ strains and recipient FA2-2 (Rif^r Fus^r) were used. Broth matings were carried out as described in Materials and Methods. After overnight incubation of the selective plates at 37°C, the hemolytic colonies (transconjugants) were randomly selected from each plate and purified, and the drug resistance of the transconjugants was examined. Of the 58 strains, 32 (55%) transferred the hemolysin marker and are shown in this table.

[b] Transconjugants which expressed representative phenotypes were marked from a to g, and one of each was used for a subsequent second mating, as shown in Table 4.

specifically, 8 students had *E. faecalis*, 15 had both *E. faecalis* and *E. faecium*, 69 had *E. faecium*, and 8 had *S. avium* in fecal specimens.)

***E. faecalis* clinical isolates mainly hemolytic.** When horse blood was used for detection of hemolysin production by the clinical isolates, 31% of the *E. faecalis* strains were hemolytic (data not shown); however, when human or rabbit blood was used, about 60% were hemolytic (Table 1). Other reports have indicated a relatively low frequency of hemolytic activity (12 to 20%) among *E. faecalis* clinical isolates when horse blood was used (9, 21). Among the 97 clinical isolates, 48 produced gelatinase. Interestingly, the hemolytic strains which produced gelatinase produced a hemolytic zone on human and rabbit blood agar but not on horse blood. (With rabbit blood, it was necessary to incubate the plates for 2 to 3 days to observe hemolysis; this time was in contrast to the required isolation time for human blood, which exhibited good hemolysis after overnight incubation.) The various sources of the clinical isolates of *E. faecalis* and the related percentages of those which were hemolytic are shown in Table 1.

About half of the clinical isolates examined were isolated from urine. Of the 50 urinary tract isolates, 25 (50%) were isolated from specimens representing pure cultures of *E. faecalis*. Among 25 samples containing mixed cultures, 11 contained *Pseudomonas aeruginosa*, 4 contained *Escherichia coli*, and the others contained a variety of other bacterial species.

The frequency of hemolysin production among *E. faecalis* strains from feces of healthy students was low (4 of 23, or 17%) compared with that of the clinical isolates. In the experiments noted below, hemolysin production on human blood agar was observed.

Apparent pheromone response by clinical isolates. To determine if strains contained a pheromone-responding plasmid, the clinical isolates obtained in this study were each exposed to a culture filtrate of plasmid-free *E. faecalis* JH2-2. Most (85%) of the hemolytic strains exhibited a clumping response (Table 2). It is noteworthy that about 90% of the hemolytic strains were resistant to one or more drugs, whereas this was true for only about 54% of nonhemolytic strains.

Transferability of hemolysin production. To determine the transferability of the hemolytic trait, mating experiments were performed in broth. Of the 58 hemolytic strains, 32 (55%) were able to donate the determinant for hemolysin production (Table 3). (Of those 32 strains, 15 were the strains that produced gelatinase and were unable to lyse horse erythrocytes.) All of these strains aggregated upon exposure to pheromone, and in most cases, transconjugants arose at about 10^{-3} per donor. Transconjugants acquiring only the hemolytic property were found in all the matings except one; the hemolytic trait in these derivatives could be transferred to another recipient (i.e., from FA2-2 to JH2SS) at a frequency of 10^{-3} to 10^{-1} per donor (data not shown).

Resistance traits were frequently transferred (unselected) with the hemolysin trait (Table 3). To determine linkage between the hemolytic property and the drug resistance of those transconjugants, one strain was selected in each case from the transconjugants which expressed the representative phenotype (the strains are indicated a to g in Table 3); the second mating experiments were performed in broth (Table 4). When the mating mixtures were plated on nonselective media, transconjugants expressing only the hemolytic trait were found in all the matings except that involving strain b. When selected on drug plates, transconjugants expressing drug resistance without the hemolytic property were found (Table 4). With strain b, the erythromycin resistance determinant was more frequently transferred than was the hemolytic determinant, suggesting that a nontransferable hemolysin plasmid was mobilized by a transferable erythromycin resistance plasmid. In none of the matings was there evidence for linkage between the hemolytic property and drug resistance.

Of the 26 strains that did not transfer the hemolysin trait in broth, 17 exhibited induced clumping upon exposure to the JH2-2 culture filtrate. The hemolysin determinant in these strains may exist on a nontransferable plasmid or on the chromosome; it is possible that the clumping responses are due to coresident plasmids. Some plasmids that do not transfer in broth mating transfer by filter mating on a solid surface (3). To determine if transfer of the hemolytic trait would occur on a solid surface, filter mating experiments were performed. Only one strain was able to donate the hemolytic determinant, at a frequency of about 10^{-5} per donor (data not shown).

TABLE 4. Separation of hemolysin production and drug resistance by conjugal transfer

Strain[a]	Phenotype of strain used for second transfer[b]	Selection on THB[c]	No. of transconjugants/ donor cell	Phenotypes of transconjugants (%)[d]
a	Hly Em		1×10^{-2}	Hly Tra^+ (10), Hly Em (90)
		+ERY	1×10^{-2}	Em Tra^+ (1), Hly Em (99)
b	Hly Em		1×10^{-6}	Hly Em
		+ERY	1×10^{-3}	Em Tra^+ (99.9), Hly Em (0.1)
c	Hly Cm		1×10^{-2}	Hly Tra^+ (10), Hly Cm (90)
		+CHLOR	1×10^{-2}	Cm Tra^- (10), Hly Cm (90)
d	Hly Km Gm		3×10^{-3}	Hly Tra^+ (25), Hly Km Gm (75)
		+KAN or GEN	3×10^{-3}	Km Gm Tra^- (0.3), Hly Km Gm (99.7)
e	Hly Em Cm Km Gm		1×10^{-2}	Hly Tra^+ (10), Hly Em Cm Km Gm (90)
		+ERY, CHLOR, KAN, or GEN	1×10^{-2}	Em Cm Km Gm Tra^+ (60), Hly Em Cm Km Gm (40)
f	Hly Em Cm		5×10^{-2}	Hly Tra^+ (5), Hly Em Cm (95)
		+ ERY or CHLOR	1×10^{-2}	Em Cm Tra^+ (10), Hly Em Cm (90)
g	Hly Km Gm		4×10^{-2}	Hly Tra^+ (10), Hly Km Gm (90)
		+ KAN or GEN	1×10^{-2}	Km Gm Tra^+ (40), Hly Km Gm (60)

[a] Strains were derived from the transconjugants shown in Table 3.
[b] Host of donor strain was FA2-2 (Rif^r Fus^r).
[c] Recipient strain was JH2SS (Str^r Spc^r). Selective agar plates were Todd-Hewitt broth agar (THB) containing 4% human blood, Streptomycin (500 μg/ml), Spectinomycin (500 μg/ml) plus drug indicated. ERY, Erythromycin; CHLOR, chloramphenicol; KAN, kanamycin; GEN, gentamicin.
[d] Tra, Transferability and clumping response. Transferability was tested by a third transfer from JH2SS to FA2-2 in a broth mating.

DISCUSSION

Although the isolates of our study came from only two hospitals, it is likely that they represent typical *E. faecalis* strains found in human infections in Japan. This supposition is supported by general observations of pathogenic microorganisms in Gunma prefecture (T. Tanaka, personal communication) and among clinical isolates from Yoikuin Hospital in Tokyo (K. Shimada, personal communication). In these studies, the specimens in which *E. faecalis* were distributed were almost the same as those presented here.

Most streptococcal group D parenteral infections have been found to involve *E. faecalis*, and more than half were hemolytic as defined by their appearance on human blood agar. Facklam (12) has reported that most parenteral group D infections analyzed at the Centers for Disease Control in Atlanta, Ga., were caused by *E. faecalis*, but a lower percentage (11%) of these strains were hemolytic. It should be noted, however, that in that study, hemolysin was determined on rabbit blood agar.

The situation with fecal specimens from healthy individuals is different from that with patients with parenteral infections; a much lower percentage (17%) of *E. faecalis* strains were hemolytic. (*E. faecium* was found to be a more common group D inhabitant of the gut of healthy adults, an observation also noted by Unsworth [24].) A similar contrast in hemolysin expression between fecal and parenteral strains has been reported for *Escherichia coli* (7, 8, 20). Most hemolytic *E. faecalis* strains exhibited a clumping response when exposed to a culture filtrate of the plasmid-free strain JH2-2, and in over half of the isolates, the hemolysin trait could be transferred conjugatively in broth. The data are consistent with a previous report (9) showing that hemolytic clinical isolates from patients in Ann Arbor, Mich., were significantly more likely to exhibit a clumping response than were nonhemolytic strains. Most of our isolates were resistant to one or more antibiotics, and in many cases, resistance traits were transferred (unselected) with the hemolysin trait. However, direct linkage between the hemolysin determinant and resistance determinants was not evident.

Hemolysin determinants in *E. faecalis* usually exist on plasmids (3), and comparison of several determinants showed strong homology (19). The hemolysin plasmids pAD1, pAMγ1, pJH2, and pOB1 all confer a pheromone response, and hybridization studies have found them to be closely related (19). pAD1 and pAMγ1 are almost identical, and both respond to the peptide pheromone cAD1 (6, 19). The conjugative hemolysin plasmid pPD5, which transfers only on a filter membrane, does not appear to confer a pheromone response (25). In the present study, only one of those hemolytic strains that did not transfer the hemolytic trait in broth transferred it in filter matings. In general, *E. faecalis* plasmids that require mating on solid surfaces transfer at frequencies on the order of 10^{-4} per donor. It is possible that among the strains found to be unable to transfer the hemolytic trait, even in filter matings, some have the determinant located on the chromosome.

Together with the previous 50% lethal dose data in mice that show a significant contribution of hemolysin to virulence (17), the epidemiological data presented here suggest that the hemolysin of *E. faecalis* may play a role in human infections. It appears that, in most cases, the hemolysin determinant can be transferred at relatively high frequencies and is therefore probably plasmid borne. In its natural environment, the bacteriocin activity associated with the hemolysin probably contributes to self-selection. That is, nearby nonhemolytic (i.e., bacteriocin-sensitive) strains may be killed if they do not acquire the plasmid. Finally, the high incidence of multiple drug resistance in hemolytic strains raises the possibility that conjugative hemolysin plasmids might play a significant role in the dissemination (mobilization) of the resistance determinants.

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LITERATURE CITED

1. **Basinger, S. F., and R. W. Jackson.** 1968. Bacteriocin (hemolysin) of *Streptococcus zymogenes*. J. Bacteriol. **96**:1895–1902.
2. **Brock, T. D., and J. M. Davie.** 1963. Probable identity of a group D hemolysin with a bacteriocine. J. Bacteriol. **86**:708–712.

3. **Clewell, D. B.** 1981. Plasmids, drug resistance, and gene transfer in the genus *Streptococcus*. Microbiol. Rev. **45**:409–436.
4. **Clewell, D. B., and B. L. Brown.** 1980. Sex pheromone cAD1 in *Streptococcus faecalis*: induction of a function related to plasmid transfer. J. Bacteriol. **143**:1063–1065.

4a. **Clewell, D. B., E. Ehrenfeld, R. Kessler, Y. Ike, A. Franke, M. Madion, J. Shaw, R. Wirth, F. An, M. Mori, C. Kitada, M. Fujino, and A. Suzuki.** 1986. Sex pheromone systems in *Streptococcus faecalis*. Banbury Rep. **24**:131–142.

5. **Clewell, D. B., P. K. Tomich, M. C. Gawron-Burke, A. E. Franke, Y. Yagi, and F. Y. An.** 1982. Mapping of *Streptococcus faecalis* plasmids pAD1 and pAD2 and studies relating to transposition of Tn*917*. J. Bacteriol. **152**:1220–1230.
6. **Clewell, D. B., Y. Yagi, Y. Ike, R. A. Craig, B. L. Brown, and F. An.** 1982. Sex pheromones in *Streptococcus faecalis*: multiple pheromone systems in strain DS5, similarities of pAD1 and pAMγ1, and mutants of pAD1 altered in conjugative properties, p. 97–100. *In* D. Schlessinger (ed.), Microbiology—1982. American Society for Microbiology, Washington, D.C.
7. **Cook, E. M., and S. P. Ewins.** 1975. Properties of strains of *Escherichia coli* isolated from a variety of sources. J. Med. Microbiol. **8**:107–111.
8. **Dudgeon, L. S., E. Wordley, and F. Bawtree.** 1921. On bacillus coli infections of the urinary tract, especially in relation to haemolytic organisms. J. Hyg. **20**:127–164.
9. **Dunny, G. M., B. L. Brown, and D. B. Clewell.** 1978. Induced cell aggregation and mating in *Streptococcus faecalis*: evidence for a bacterial sex pheromone. Proc. Natl. Acad. Sci. USA **75**:3479–3483.
10. **Dunny, G. M., and D. B. Clewell.** 1975. Transmissible toxin (hemolysin) plasmid in *Streptococcus faecalis* and its mobilization of a noninfectious drug resistance plasmid. J. Bacteriol. **124**:784–790.
11. **Dunny, G. M., R. A. Craig, R. Carron, and D. B. Clewell.** 1979. Plasmid transfer in *Streptococcus faecalis*. Production of multiple sex pheromones by recipients. Plasmid **2**:454–465.
12. **Facklam, R. R.** 1972. Recognition of group D streptococcal species of human origin by biochemical and physiological tests. Appl. Microbiol. **23**:1131–1139.
13. **Franke, A. E., and D. B. Clewell.** 1980. Evidence for conjugal transfer of a *Streptococcus faecalis* tansposon (Tn*916*) from a chromosomal site in the absence of plasmid DNA. Cold Spring Harbor Symp. Quant. Biol. **45**:77–80.
14. **Granato, P. A., and R. W. Jackson.** 1969. Bicomponent nature of lysin from *Streptococcus zymogenes*. J. Bacteriol. **100**:865–868.
15. **Ike, Y., and D. B. Clewell.** 1984. Genetic analysis of the pAD1 pheromone response in *Streptococcus faecalis*, using transposon Tn*917* as an insertional mutagen. J. Bacteriol. **158**:777–783.
16. **Ike, Y., R. A. Craig, B. A. White, Y. Yagi, and D. B. Clewell.** 1983. Modification of *Streptococcus faecalis* sex pheromones after acquisition of plasmid DNA. Proc. Natl. Acad. Sci. USA **80**:5369–5373.
17. **Ike, Y., H. Hashimoto, and D. B. Clewell.** 1984. Hemolysin of *Streptococcus faecalis* subspecies *zymogenes* contributes to virulence in mice. Infect. Immun. **45**:528–530.
18. **Jacob, A. E., and S. J. Hobbs.** 1974. Conjugal transfer of plasmid-borne multiple antibiotic resistance in *Streptococcus faecalis* var. *zymogenes*. J. Bacteriol. **117**:360–372.
19. **LeBlanc, D. J., L. N. Lee, D. B. Clewell, and D. Behnke.** 1983. Broad geographical distribution of a cytotoxin gene mediating beta-hemolysis and bacteriocin activity among *Streptococcus faecalis* strains. Infect. Immun. **40**:1015–1022.
20. **Minshew, B. H., J. Jorgensen, G. W. Counts, and S. Falkow.** 1978. Association of hemolysin production, hemagglutination of human erythrocytes, and virulence for chicken embryos of extraintestinal *Escherichia coli* isolates. Infect. Immun. **20**:50–54.
21. **Parker, M. T., and L. C. Ball.** 1976. Streptococci and aerococci associated with systemic infection in man. J. Med. Microbiol. **9**:275–302.
22. **Tomich, P. K., F. Y. An, and D. B. Clewell.** 1980. Properties of erythromycin-inducible transposon Tn*917* in *Streptococcus faecalis*. J. Bacteriol. **141**:1366–1374.
23. **Tomich, P. K., F. Y. An, S. P. Damle, and D. B. Clewell.** 1979. Plasmid-related transmissibility and multiple drug resistance in *Streptococcus faecalis* subsp. *zymogenes* strain DS16. Antimicrob. Agents Chemother. **15**:828–830.
24. **Unsworth, P. F.** 1979. Streptococci in human faeces, p. 239–240. *In* M. T. Parker (ed.), Pathogenic streptococci. Reedbooks, Ltd., Surrey, United Kingdom.
25. **Yagi, Y., R. Kessler, J. Shaw, D. Lopatin, F. An, and D. Clewell.** 1983. Plasmid content of *Streptococcus faecalis* strain 39-5 and identification of a pheromone (cPD1)-induced surface antigen. J. Gen. Microbiol. **129**:1207–1215.