Journal of Food Protection, Vol. 56, No. 6, Pages 489-492 (June 1993)

Antibiotic Resistance Among Enterococcal Isolates from Environmental and Clinical Sources

LINDA M. KNUDTSON and PAUL A. HARTMAN*

Department of Microbiology, Immunology and Preventive Medicine, Iowa State University, Ames, Iowa 50011

(Received for publication November 20, 1992)

ABSTRACT

Antibiotic resistance among enterococci and fecal streptococci was examined by testing 149 isolates from pork, water, and clinical material, as well as 50 strains of 13 known species, for resistance to 27 different antimicrobial agents. Tests were performed by using the MicroScan Pos MIC type 6 panels. Pork isolates exhibited less resistance than either water or clinical isolates to most antibiotics, although a larger proportion of pork isolates than others was resistant to tetracycline. Comparisons of antimicrobial-resistance patterns between enterococcal species revealed that *Enterococcus faecium* was most resistant to β-lactam antimicrobials, especially ampicillin, whereas *Enterococcus faecalis* seemed to be the most resistant to the synergistic effects of antimicrobial combinations. Vancomycin resistance was observed in one *Enterococcus hirae* isolate from water. Enterococcal isolates from any of the sources tested did not show multiple resistance to antibiotics (such as gentamicin, ampicillin, streptomycin, and vancomycin) used to treat serious infections caused by gram-positive cocci.

Recent attention has focused on enterococci because of their remarkable and increasing resistance to antimicrobial agents (*13*). This resistance allows them to survive in environments in which antimicrobial agents are heavily used. Antimicrobials are given to food animals, such as swine, to improve their growth rate and feed conversion. However, many people believe that the use of antimicrobials in humans or animals is often followed by appearance of resistant microorganisms (*14*). Studies by Cohen and Tauxe (*2*) suggested that the antimicrobial drugs to which food animals were exposed provided selective pressure that led to the appearance and persistence of drug-resistant strains. Specifically, these authors associated the occurrence of certain drug-resistant *Salmonella* sp. to antimicrobial use in food animals. If antimicrobial use in food animals is linked to antimicrobial resistance in *Salmonella*, *Enterococcus* species in the intestinal tract might also be expected to develop similarly elevated resistance patterns.

Antimicrobial resistance in enterococci can be divided into two general types, intrinsic and acquired. Intrinsic resistance is present in all or most strains of those species, and the genes appear to reside on the chromosome. Intrinsic resistance includes resistance to semisynthetic, penicillinase-resistant penicillins, cephalosporins, and low levels of aminoglycosides and clindamycin. Acquired resistance results from a mutation in cellular DNA or acquisition of new DNA. Examples of acquired resistance include resistance to chlorampenicol, erythromycin, tetracycline, high levels of aminoglycosides and clindamycin, penicillin by means of penicillinase, fluoroquinolones, and vancomycin (*13*). Several researchers have shown that differences in antimicrobial susceptibility exist between *Enterococcus faecium* and *Enterococcus faecalis* (*4,10,11*). The possible existence of similar susceptibility differences among the more recently described species of *Enterococcus* needs to be determined. An assessment of possible correlations between a certain species and a given susceptibility pattern could provide valuable information (*15*). Few data are available on antimicrobial-resistance patterns of isolates from widely divergent environmental sources studied in a single laboratory by using a common procedure.

In this study, enterococci and fecal streptococci were collected from three sources: pork, including pork carcasses and processed pork products; water, including samples from rivers, lakes, and wells; and clinical material from several sources. These samples, as well as known strains, were tested for resistance to 24 antimicrobials and 3 synergy screens with MicroScan Pos MIC type 6 panels. The results were examined for differences in resistance patterns between enterococci from the three different sources (pork, water, clinical). Differences in resistance patterns between different species of enterococci and fecal streptococci also were examined.

MATERIALS AND METHODS

Cultures

Fifty named strains of 13 species of enterococci and *Streptococcus bovis* and *Streptococcus equinus* were collected from various sources, and their identities were confirmed (*7*). Cultures were also isolated from pork carcasses during slaughter and from fresh and spoiled pork products. All pork sample cultures were isolated according to Knudtson and Hartman (*8*). Enterococci from water samples (*6*) were isolated by the membrane filter method (*1*). Clinical isolates were collected from patients at one hospital over a 2-year period (*6*). Primary isolation on blood agar plates revealed colonies that comprised gram-positive cocci. Catalase, bile esculin, and Lancefield grouping tests confirmed that the isolates were group D enterococci. Species identifications were carried out as indicated by Knudtson and Hartman (*7*).

Antibiotic susceptibility testing

Inoculum preparation for the MicroScan Pos MIC type 6 panels (Baxter Diagnostics, Deerfield, IL) was performed by using the log-phase technique specified by the manufacturer. Panels were inoculated, covered, and incubated at 37°C. After 18 to 24 h of incubation, sensitivity or resistance was interpreted as indicated in the manufacturer's instructions. Gentamicin and streptomycin synergy screens consist of wells containing a high concentration of each antibiotic; no growth indicates that a high level can be used in combination with an aminoglycoside to treat infections caused by an enterococcus that is resistant to an individual antibiotic.

RESULTS AND DISCUSSION

The percentages of water, pork, and clinical isolates that were resistant to 27 different antimicrobial agents are shown in Table 1. Generally, smaller proportions of the pork isolates were resistant than either the water or clinical isolates. Only with cefazolin, imipenem, and tetracycline were larger proportions of the pork isolates resistant than of the clinical isolates. Only in three instances, with penicillin, erythromycin, and tetracycline, did higher proportions of the pork isolates exhibit resistance than did the water isolates (Table 1). Water isolates were more resistant than either the pork or clinical isolates to all cephalosporins, amikacin, gentamicin, imipenem, and rifampin. Only one isolate, from water, was resistant to vancomycin. The possibility exists that some of the water samples contain animal or human sewage runoff, which may explain some of the resistance seen in these samples.

TABLE 1. *Percentages of water, pork, and clinical isolates resistant to 27 different antimicrobial agents.*

Antibiotics tested	Water (49)[a]	Pork (50)	Clinical (50)
Aminoglycosides			
Amikacin	96[b]	48	90
Gentamicin	88	36	74
Gentamicin synergy screen	--[c]	--	36
Streptomycin synergy screen	6	--	32
Cephalosporins			
Cephalothin	59	32	38
Cefazolin	73	30	26
Cefuroxime	94	84	84
Cefotaxime	94	60	84
Ceftriaxone	92	62	76
Penicillins			
Amoxicillin/K clavulanate	--	--	2
Ampicillin	--	--	4
Ampicillin/Sulbactam	--	--	4
Oxacillin	96	84	96
*Penicillin	--	2	4
Ticarcillin/K clavulanate	94	68	98
Other β-lactams			
Imipenem	10	6	4
Miscellaneous			
Chloramphenicol	--	--	--
Ciprofloxacin	29	6	62
Clindamycin	92	86	92
*Erythromycin	35	38	62
Nitrofurantoin	--	--	--
Norfloxacin	31	2	46
Rifampin	49	20	26
Sulfamethoxazole	100	98	100
*Tetracycline	37	88	56
Trimethoprim/Sulfamethoxazole	22	--	36
Vancomycin	2	--	--

[a] Numbers in parentheses indicate number of isolates tested.
[b] Numbers indicate the percentage of strains resistant.
[c] All isolates were susceptible.
* Antibiotics approved for use in animal feeds and medicine (*12*).

It has been suggested that the animal husbandry practices of this decade have contributed to the dissemination of antibiotic resistance among intestinal isolates (*2,3,14*). Langlois et al. (*9*) stated that the selection for resistant bacteria brought about by the use of antimicrobials would not be easily reversed by partial restrictions of antibiotics because the resistant bacteria are passed on from one generation to another. Resistant fecal coliforms were present in swine at the time of slaughter, regardless of recent administration of antimicrobial agents (*9*). If this were true for enterococci, isolates from pork carcasses and processed pork should show antimicrobial-resistance patterns similar to those of the clinical isolates, but the incidence of acquired resistance generally was lower in isolates from pork than from water or clinical material. The major exception was resistance to tetracycline, which is a common additive to swine feeds (*12*).

When resistance patterns of known strains and environmental isolates were compared (Table 2), several trends could be seen. All enterococci possessed a degree of resistance to the aminoglycosides and cephalosporins, as would be predicted by their intrinsic resistance. Intrinsic resistance to the semisynthetic penicillins, ticarcillin and oxacillin, also was prevalent throughout most species of the enterococci (Table 2). *Enterococcus cecorum*, however, was not resistant to any of these antimicrobials for which it should carry intrinsic resistance. It does, however, show resistance to clindamycin along with the other enterococci (Table 2). As stated in the literature (*4,10*), *E. faecium* carries the highest amount of resistance to β-lactam antimicrobials, especially ampicillin. On the other hand, it was also stated that *E. faecium* strains are often more refractory to synergistic effects of antibiotic combinations. Our results show that *E. faecalis* was more often resistant to synergistic combinations of antibiotics. Grayson et al. (*5*) stated that *Enterococcus raffinosus* isolates showed higher levels of resistance to penicillin than *Enterococcus avium* isolates; this was supported by our findings. Resistance to vancomycin was seen only in *Enterococcus hirae*, collected from a sample of creek water.

In conclusion, pork and pork products did not harbor enterococci with levels of antibiotic resistance that were substantially higher than those possessed by enterococci obtained from water or from clinical material with the exception of resistance to tetracycline. No more resistance to antibiotics used clinically to treat gram-positive infections in humans was observed in any isolate from pork. Therefore, antibiotic-resistant enterococci and fecal streptococci of pork origin do not present an exceptional public health hazard. Clinical and water isolates carry more varied antibiotic resistance patterns than do isolates from pork. Also, antibiotic-resistance patterns differ among enterococcal species, even those isolated from environmental sources.

TABLE 2. *Percentages of resistance among enterococci and fecal streptococci.*

	E. avium(5)[a]	*E. casseliflavus*(9)	*E. cecorum*(2)	*E. durans*(9)	*E. faecalis*(81)	*E. faecium*(34)	*E. gallinarum*(7)	*E. hirae*(12)	*E. malodoratus*(11)	*E. mundtii*(6)	*E. pseudoavium*(10)	*E. raffinosus*(2)	*E. solitarius*(2)	*S. bovis*(4)	*S. equinus*(1)	Enterococci NO ID(4)
Aminoglycosides																
Amikacin	60[b]	78	50	56	88	59	71	92	45	100	10	100	50	--	--	96
Gentamicin	60	67	--	56	70	65	57	75	45	100	10	100	--	--	--	88
Gentamicin synergy	60	--	--	--	24	--	--	--	--	--	--	--	--	--	--	--
Streptomycin synergy	--[c]	--	--	--	22	6	14	--	--	--	--	50	--	--	--	6
Cephalosporins																
Cephalothin	20	22	--	56	42	62	71	50	18	50	20	50	--	--	100	59
Cefazolin	20	22	--	56	69	68	86	58	36	83	40	50	50	--	100	73
Cefuroxime	100	78	50	89	87	94	100	67	82	100	60	100	50	--	100	94
Cefotaxime	20	56	--	89	86	79	100	67	36	83	30	100	50	--	--	94
Ceftriaxone	20	44	--	78	82	82	100	67	45	85	20	100	50	--	--	92
Penicillins																
Amoxicillin/K clavulanate	--	--	--	--	--	3	--	--	--	--	--	--	--	--	--	--
Ampicillin	--	--	--	--	--	6	--	--	--	--	--	--	--	--	--	--
Ampicillin/Sulbactam	--	--	--	--	--	6	14	--	--	--	--	--	--	--	--	--
Oxacillin	100	100	--	89	93	91	100	67	100	100	80	100	100	--	100	96
Penicillin	--	--	--	--	--	9	--	--	--	--	--	50	--	--	--	--
Ticarcillin/K clavulanate	100	100	--	67	90	79	100	67	82	100	20	100	100	--	100	94
Other β-lactams																
Imipenem	--	11	--	--	1	21	14	17	9	--	--	--	--	--	--	10
Miscellaneous																
Chloramphenicol	--	--	--	--	2	--	--	--	--	--	--	--	--	--	--	--
Ciprofloxacin	80	56	--	22	40	35	29	8	27	33	--	--	--	75	--	29
Clindamycin	80	100	100	78	93	88	86	92	73	100	60	100	100	--	100	92
Erythromycin	40	33	100	44	46	53	14	8	36	33	40	50	--	--	--	35
Nitrofurantoin	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Norfloxacin	60	33	--	22	33	21	29	17	27	67	--	--	--	100	--	31
Rifampin	--	22	--	11	28	53	29	8	9	--	--	50	50	--	100	49
Sulfamethoxazole	100	100	100	100	95	100	100	100	100	100	70	100	100	100	100	100
Tetracycline	60	33	--	67	64	35	71	8	73	--	60	50	50	--	--	37
T/S[d]	40	22	50	22	24	24	100	8	--	17	--	--	50	50	--	22
Vancomycin	--	--	--	--	--	--	--	8	--	--	--	--	--	--	--	--

[a] Indicates number of strains tested.
[b] Numbers indicate the percentage of strains resistant.
[c] All isolates were susceptible.
[d] T/S = trimethoprim/sulfamethoxazole.

ACKNOWLEDGMENT

Supported by a USDA grant to the Food Safety Consortium. Journal Paper No. J-15122 of the Iowa Agriculture and Home Economics Experiment Station, Ames, project 2991.

REFERENCES

1. American Public Health Association. 1985. Standard methods for the examination of water and wastewater, 16th ed. American Public Health Association, Washington, DC.
2. Cohen, M. L., and R. V. Tauxe. 1986. Drug-resistant *Salmonella* in the United States: An epidemiological perspective. Science 234:964-969.
3. DuPont, H. L., and J. H. Steele. 1987. Use of antimicrobial agents in animal feeds: Implications for human health. Rev. Infect. Dis. 9:447-460.
4. Gray, J. W., D. Stewart, and S. J. Pedler. 1991. Species identification and antibiotic susceptibility testing of enterococci isolated from hospitalized patients. Antimicrob. Agents Chemother. 35:1943-1945.
5. Grayson, M. L., G. M. Eliopoulos, C. B. Wennersten, K. L. Rouff, K. Klimm, F. L. Sapico, A. S. Bayer, and R. C. Moellering, Jr. 1991. Comparison of *Enterococcus raffinosus* with *Enterococcus avium* on the basis of penicillin susceptibility, penicillin-binding protein analysis, and high-level aminoglycoside resistance. Antimicrob. Agents Chemother. 35:1408-1412.
6. Knudtson, L. M. 1992. Classification of enterococci and their roles in spoilage of pork products and as sanitary indicators in pork processing. Ph.D. dissertation. Iowa State University, Ames.
7. Knudtson, L. M., and P. A. Hartman. 1992. Routine procedures for isolation and identification of enterococci and fecal streptococci. Appl. Environ. Microbiol. 58:3027-3031.
8. Knudtson, L. M., and P. A. Hartman. 1993. Enterococci in pork

processing. J. Food Prot. 56:6-9.
9. Langlois, B. E., K. A. Dawson, I. Leak, and D. K. Aaron. 1988. Antimicrobial resistance of fecal coliforms from pigs in a herd not exposed to antimicrobial agents for 126 months. Vet. Microbiol. 18:147-153.
10. Louie, M., A. E. Simor, S. Szeto, M. Patel, B. Kreiswirth, and D. E. Low. 1992. Susceptibility testing of clinical isolates of *Enterococcus faecium* and *Enterococcus faecalis*. J. Clin. Microbiol. 30:41-45.
11. Moellering, R. C., Jr., O. M. Korzeniowski, M. A. Sande, and C. B. Wennersten. 1979. Species-specific resistance to antimicrobial synergism in *Streptococcus faecium* and *Streptococcus faecalis*. J. Infect. Dis. 140:203-208.
12. Muirhead, S. (ed.). 1992. 1993 Feed additive compendium. Miller Publishing Co., Minnetonka, MN.
13. Murray, B. E. 1990. The life and times of the *Enterococcus*. Clin. Microbiol. Rev. 3:46-65.
14. Neu, H. C. 1992. The crisis in antibiotic resistance. Science 257:1064-1072.
15. Rouff, K. L. 1990. Recent taxonomic changes in the genus *Enterococcus*. Eur. J. Clin. Microbiol. Infect. Dis. 9:75-79.

Crandall and Montville, *cont. from p. 488*

megadalton plasmid in *Pediococcus pentosaceus* with bacteriocin activity. Appl. Environ. Microbiol. 50:1538-1541.
7. Daniels, R. W. 1991. Applying HACCP to new-generation refrigerated foods at retail and beyond. Food Technol. 45(6):122, 124.
8. Dezfulian, M., and J. G. Bartlett. 1984. Detection of *Clostridium botulinum* type A toxin by enzyme-linked immunosorbent assay with antibodies produced in immunologically tolerant animals. J. Clinical Microbiol. 19:645-648.
9. Eklund, M. W. 1982. Significance of *Clostridium botulinum* in fishery products preserved short of sterilization. Food Technol. 36:107-112, 115.
10. Federal Register. 1988. Nisin preparation: Affirmation of GRAS status as a direct human food ingredient. 21CFR Part 184. 53:11247-11251.
11. Food and Drug Administration. 1984. The bacteriological analytical manual, 6th ed. Association of Official Analytical Chemists, Arlington, VA.
12. Greenberg, R. A., R. B. Tompkin, B. O. Bladel, R. S. Kittaka, and A. Anellis. 1966. Incidence of mesophilic *Clostridium* spores in raw pork, beef, and chicken in processing plants in the United States and Canada. Appl. Microbiol. 14:789-793.
13. Huhtanen, C. N., R. C. Whiting, A. J. Miller, and J. E. Call. 1992. Qualitative correlation of the mouse neurotoxin and enzyme-linked immunoassay for determining *Clostridium botulinum* types A and B toxins. J. Food Safety 12:119-127.
14. Hutton, M. T., P. A. Chehak, and J. H. Hanlin. 1991. Inhibition of botulinum toxin production by *Pediococcus acidilactici* in temperature abused refrigerated foods. J. Food Safety 11:255-267.
15. Klaenhammer, T. R. 1988. Bacteriocins of lactic acid bacteria. Biochemie. 10:337-349.
16. Lamanna, C., and J. P. Lowentahl. 1951. The lack of identity between hemagglutinin and the toxin of type A botulinal organism. J. Bacteriol. 61:751-752.
17. Lerke, P., and L. Farber. 1971. Heat pasteurization of crab and shrimp from the pacific coast of the United States: public health aspects. J. Food Sci. 36:277-279.
18. Lewus, C. B. 1991. Characterization of bacteriocins produced by lactic acid bacteria isolated from meat. Ph. D. dissertation. Rutgers, the State University. New Brunswick, NJ.
19. Lewus, C. B., A. Kaiser, and T. J. Montville. 1991. Inhibition of food-borne bacterial pathogens by bacteriocins from lactic acid bacteria isolated from meat. Appl. and Environ. Microbiol. 57:1683-1688.
20. Lynt, R. K., D. A. Kautter, and H. M. Solomon. 1982. Differences and similarities among proteolytic and nonproteolytic strains of *Clostridium botulinum* types A, B, E and F: a review. J. Food Prot. 45:466-474.
21. Notermans, S., J. Dufrenne, and B. M. Lund. 1990. Botulism risk of refrigerated, processed foods of extended durability. J. Food Prot. 53:1020-1024.
22. Okereke, A., and T. J. Montville. 1991. Bacteriocin inhibition of *Clostridium botulinum* spores by lactic acid bacteria. J. Food Prot. 54:349-353.
23. Okereke, A., and T. J. Montville. 1991. Bacteriocin-mediated inhibition of *Clostridium botulinum* spores by lactic acid bacteria at refrigeration and abuse temperatures. Appl. Environ. Microbiol. 57:3423-3428.
24. Refrigerated Foods and Microbiological Criteria Committee of the National Food Processors Association. 1988. Factors to be considered in establishing good manufacturing practices for the production of refrigerated foods. Dairy Food Sanit. 8:288-291.
25. Scott, V. N., and D. T. Bernard. 1982. Heat resistance of spores of non-proteolytic type B *Clostridium botulinum*. J. Food Prot. 45:909-912.
26. Simunovic, J., J. L. Oblinger, and J. P. Adams. 1985. Potential for growth of nonproteolytic types of *Clostridium botulinum* in pasteurized restructured meat products: a review. J. Food Prot. 48:265-276.
27. Smelt, J. P. P. M., and H. Haas. 1978. Behaviour of proteolytic *Clostridium botulinum* types A and B near the lower temperature limits of growth. Eur. J. Appl. Microbiol. Biotechnol. 5:143-154.
28. Smith, G. R., and A. M. Young. 1980. *Clostridium botulinum* in British soil. J. Hyg. 85:271-274.
29. Solomon, H. M., D. A. Kautter, and R. K. Lynt. 1982. Effect of low temperatures on growth of nonproteolytic *Clostridium botulinum* types B and F and proteolytic type G in crabmeat and broth. J. Food Prot. 45:516-518.
30. Solomon, H. M., R. K. Lynt, T. Lilly, Jr., and D. A. Kautter. 1977. Effect of low temperatures on growth of *Clostridium botulinum* spores in meat of the blue crab. J. Food Prot. 40:5-7.
31. Somers, E., and B. R. DasGupta. 1991. *Clostridium botulinum* types A, B, C_1, and E produce proteins with or without hemagglutinating activity: do they share common amino acid sequences and gene? J. Prot. Chem. 10:415-425.
32. Somers, E. B., and S. L. Taylor. 1987. Antibotulinal effectiveness of nisin in pasteurized process cheese spreads. J. Food Prot. 50:842-848.
33. Sperber, W. H. 1982. Requirements of *Clostridium botulinum* for growth and toxin production. Food Technol. 36(12):89-94.
34. Tanaka, N., E. Traisman, M. H. Lee, R. G. Cassens, and E. M. Foster. 1980. Inhibition of botulinum toxin formation in bacon by acid development. J. Food Prot. 43:450-457.
35. Van Garde, S. J., and M. J. Woodburn. 1987. Food discard practices of householders. J. Am. Dietet. Assoc. 87:322-329.
36. Wyatt, L. D. and V. Guy. 1980. Relationships of microbial quality or retail meat samples and sanitary conditions. J. Food Prot. 43:385-389.