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RISK MANAGEMENT ON THE FARM AND IN THE ABATTOIR

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In recent years food safety has been encouraged at the primary production stage through advice and mandated during food processing by legislation. On the farm, several pre-harvest interventions such as competitive inhibition, reduction in hide soiling, vaccination as well as diet manure and slurry management have been explored to control pathogens like *Escherichia coli* O157. During slaughter chemical dehairing, hide decontamination and carcass decontamination activities have been researched. The objective of this paper is to present an short review of these suggested controls in a risk management framework with *E. coli* O157 as the target pathogen.

INTRODUCTION

Bovine spongiform encephalopathy (first reported in 1986) and to a lesser extent the well publicised *Escherichia coli* O157:H7 outbreak in Lanarkshire in Scotland focused attention on food safety. As a consequence of the associated media interest and hype, bacterial pathogens like *Salmonella* have become household names and are of keen consumer focus. Consumer demand in turn resulted in government action and authorities responsible for food safety have been established in most European countries as has the European Food Safety Authority (EFSA). Considerable research resources have also been directed at food safety risk analysis. Risk assessments have been reported for a variety of bacterial pathogens in associated food products. Risk management systems such as hazard analysis and critical control point (HACCP) have been legally mandated and risk communicators have spent millions of euro on consumer food safety education and motivation. Despite this investment of resources, nobody would suggest that the contamination of food with pathogens has been successfully understood and controlled. Using *E. coli* O157 as the target pathogen, this paper will discuss risk management on farms and in the abattoir and conclude that while the former is difficult to achieve the latter may prohibitively expensive. The key to successful risk management at present lies therefore, not in a lack of effective intervention technologies, but in the absence of a suitable cost structure.

PRE-HARVEST INTERVENTIONS

Many cases of human illness caused by *E. coli* O157 have been traced to beef products or other foods or water contaminated with cattle faeces [Chapman *et al*., 1993; Hancock *et al*., 1997]. Thus the farm is the ultimate source of these pathogens. Eradication would seem to be futile for an agent that is ubiquitous and not host specific. Efforts to remove infected animals, for example, would seem pointless for an agent with a short detectable infection period and which survives well in the environment. Furthermore, animal testing would be complicated by the fact that non-carriage animals may have contaminated hides, which are the primary source of bacterial contamination on carcasses.

However, simulation models have predicted that pre-harvest reductions of *E. coli* O157 prevalence in cattle would result in substantial reductions in the contamination of beef [Jordan *et al*., 1999; USDA:FSIS 2000] and consequent human disease [USDA:FSIS 2000]. These models have been borne out in several studies, which demonstrate a moderately high correlation between prevalence of *E. coli* O157 in cattle (faeces and hide) at slaughter plants and carcass contamination rates [Elder *et al*., 2000].

The seasonal pattern in human infection has been attributed to growth and multiplication of the pathogen in feed and water. If the rates of human illness decline in Winter due to the cyclical decline in prevalence in the reservoir, this suggest that controlling *E. coli* O157 in feed and water could be effective preharvest interventions to reduce human illness.

Cattle are exposed to faecal organisms in feed and water. Lynn *et al*. [1998] reported *E. coli* concentrations of up to 10^4 per gram in feed mixes fed directly to cattle. Considering that a high yielding dairy cow will consume 35 kg or more or feed per day, at 10^4 per gram the total intake of generic *E. coli* from feed would be 3.5×10^8. A recent US study by Hancock *et al*. [2001] reported *E. coli* O157 in 1.8% of cattle feeds and 3.8% of water troughs. Several on-farm pre-harvest controls have been suggested including competitive inhibition, reductions in hide soiling, vaccination, diet, manure and slurry management and other controls.

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Competitive inhibition. In competitive inhibition, animals are fed or orally inoculated with bacteria that compete with the target organism. This approach has been demonstrated in poultry [Nisbet, 1993] and Zhao *et al*. [1008] reported similar work in cattle, although a definite product is not yet available. If competitive exclusion only reduced the incidence of carriage and shedding by 50%, this would have a significant impact on the ecology of *E. coli* O157 and environmental exposure.

Reductions in hide soiling

As hides are the primary source of bacterial contamination on beef carcasses, several countries have introduced 'clean cattle policies'. However, at least 3 studies have cast doubt on the potential effectiveness of such a policy. Van Donkersgoed *et al*. [1997] found little correlation between visible soiling and carcass bacterial counts. Jordan *et al*. [1999] estimated that, based on the best available information, the effects of an industry wide reduction in visible hide soiling would be small. Byrne *et al*. [2000] found that power-hosing the hide for 3 minutes significantly reduced *E. coli* O157 counts on hides but did not decrease carcass contamination levels.

Vaccination

There are currently no food-borne human pathogens controlled by vaccination of the animal source. Vaccination therefore represents a novel approach to controlling the risks associated with *E. coli* O157. To date at least two such vaccines have been developed in the USA by the United States of America – Agricultural Research Service (USDA-ARS) and by the University of British Columbia. The former was unsuccessful, possibly due to interference with the bovine immune system by *E. coli* O157, while the latter reduced shedding but did not prevent carriage. Both were based on the live organism.

Diet

Diez-Gonzalez *et al*. [1998] and Russell *et al*. [2000] proposed switching cattle from a grain-fed diet to an all hay diet several days prior to slaughter as this would lower levels of organic acid in the colon, selecting for *E. coli* strains that are less acid resistant. In theory, if these strains were subsequently ingested by humans they would not survive the human gastric barrier. However, an all hay diet may increase the average duration of *E. coli* O157 shedding [Hancock *et al*., 2001]. Furthermore an all hay diet would result in reduced volatile fatty acids which are inhibitory to other pathogens such as *Salmonella*.

Cattle on grain based diets shed greater numbers of the pathogen and switching from grain to forage based diets could reduce *E. coli* O157 and other Enterohemorrhagic *Escherichia coli* (EHEC) [Callaway *et al*., 2003]. Corn silage or barley increased the risk of EHEC shedding while feeding whole cottonseed had the opposite effect [Hancock *et al*., 1994; Herriot *et al*., 1998; Buchko *et al*., 2000]. Furthermore, *E. coli* O157 recovered from the faeces of grain-fed cattle were 1000-fold more resistant to extreme acid shock (such as that encountered in the human stomach) as compared to cattle fed only hay [Diez-Gonzalez *et al*., 1998]. When cattle were abruptly switched from a 90% grain finishing ration to a 100% hay diet, faecal coliform populations decreased 1000-fold and the population resistant to extreme acid shock declined 100.000 fold within 5 days [Diez-Gonzalez *et al*., 1998].

Feed withdrawal or starvation during transport increased the total *E. coli* and Enterobacter populations throughout the intestinal tract [Buchko *et al*., 2000] including an increase in *E. coli* O157 in the rumen. Fasting animals are more susceptible to colonization with pathogenic *E. coli* [Cray *et al*., 1998] and in some cases previously negative animals become positive when feed is removed for any period of time [Kudva *et al*., 1997].

Manure and slurry management

Manure and slurry management practices may also influence the prevalence of *E. coli* pathogens on farms. Data from several sources indicate that *E. coli* O157 may persist for long periods of time in manure [Wang *et al*., 1996; Bolton *et al*., 1999; Maule, 2000]. Kudva *et al*. [1998] recovered *E. coli* O157 from an ovine manure pile after 21 months. Survival of *E. coli* O157 was observed in soil cores containing rooted grass for 130 days [Maule, 2000]. However, manure slurry containing *E. coli* O157 spread onto arable land and pasture land lost viability with less that 1% recoverable after 29 days. *E. coli* O157 population decreased in pasture soil by 4 to 5 log units after 50 days but the pathogen was still detectable after 99 days [Bolton *et al*., 1999].Composting of manure at temperatures as low as 45°C will rapidly kill *E. coli* O157.

Other controls

Research by Sargeant *et al*. [2004] suggested that the removal of cats from the animal production environment would significantly decrease *E. coli* O157 carriage rates in cattle. Nielsen *et al*. [2002] suggest that non-organic bedding such as sand had lower *E. coli* O157 prevalence as compared to animals on organic materials including sawdust.

SLAUGHTER AND PROCESSING CONTROLS

A study of the prevalence of *E. coli* O157 in faeces, hides and carcasses of cattle at processing plants in the late Summer months found that 28%, 11% and 45% were contaminated respectively. Interventions on the slaughter line reduced the latter to 20% post evisceration and 2% post all interventions. Hides are the major source of carcass contamination [Bell, 1997; Small *et al*., 2000; Barkocy-Gallagher *et al*., 2003; Rivera-Betancourt *et al*., 2004] and the first potential intervention targets the hide.

Chemical dehairing

There are conflicting reports as to the effectiveness of chemical dehairing in reducing subsequent carcass contamination levels. Schnell *et al*. [1995] reported that the dehairing process resulted in visually cleaner carcasses, but the bacterial load on carcasses were not significantly reduced. In contrast, both Castillo *et al*. [1998] and Nou *et al*. [2003] reported reduced aerobic bacterial, coliform and *E. coli* counts. Data from the latter also implied that any hide intervention process incorporated into beef processing procedures would significantly reduce carcass contamination by *E. coli* O157.

Hide decontamination

Bosilevac *et al*. [2004] have reported that cetylpyrimidinium chloride (CPC), a common oral antimicrobial that has been used to decontaminate poultry carcasses, was effective at reducing microbial populations on cattle hides. The same authors tested the potential of a combined water wash and CPC treatment under conditions simulating a hide wash cabinet and concluded that a water wash followed by an antimicrobial treatment, such as CPC, held great potential as an effective hide decontamination step. Sodium hydroxide (1.6%), trisodium phosphate (4%), chlorofoam (4%) and phosphoric acid (4%) have also been approved for use in beef processing. Bosilevac *et al*. [2005] found that phosphoric acid was the most effective hide decontaminant and 500ppm chlorine was the most effective rinse. They also reported that ozonated water and electrolysed water reduced the incidence of *E. coli* O157 on bovine hides from 89% to 31% and 82% to 35%, respectively.

Carcass decontamination

Beef carcass contamination can be reduced by applying such prerequisite activities as rodding, tying and/or bagging of the bung and carcass trimming. Steam vacuuming, hot water washing, steam pasteurisation, organic acids and irradiation will reduce bacterial contamination. During rodding the oesophagus is separated from the trachea and a crocodile or plastic clip is placed at the end of the oesophagus to prevent leakage. Tying or bagging the bung is a procedure used to prevent leakage from the rectum and may reduce bacterial contamination on carcasses if performed properly [Nesbakken *et al*., 1994; Sheridan, 1998; Bolton *et al*., 2001].

Steam vacuuming is an alternative to knife trimming that effectively removes visible contamination without product loss [Dorsa *et al*., 1996a; Dorsa *et al*., 1997] and has been implemented in most US beef plants at several stages along the slaughter-line. However, this technique cannot be efficiently applied to the entire carcass [Dorsa *et al*., 1997].

Hot water [Dorsa *et al*., 1996b; Gill *et al*., 1999] and steam [Gill & Bryant, 1997; Nutsch *et al*., 1998] may be applied to the carcasses to reduce the bacterial counts. These technologies are particularly effective against gram-negative pathogens such as *E. coli*. Organic acids are widely used in the USA and there are varying reports in the literature on their decontaminating effect [Snijders *et al*., 1985; Prasai *et al*., 1991; Avens *et al*., 1996]. Lactic acid is the most commonly used organic acid and is often used in combination with hot water or steam treatments. Indeed, because none of these interventions are 100% effective, beef processors often use multiple hurdles intervention systems at various processing stages to ensure the safety of their products [Bacon *et al*., 2000].

Ionizing radiation has been approved for use in the USA for treating refrigerated or frozen uncooked meat. Traditionally performed by irradiating large lots of cuts or ground beef, the effectiveness of this treatment is well established. More recently, low dose, low penetration electron beam technology has evolved to allow whole carcass treatment. This technology, when applied in conjunction with the multiple hurdles discussed above, has the potential to eliminate most bacterial pathogens on meat carcasses including *E. coli* O157.

CONCLUSION

Control on the farm may be difficult given the ubiquitous nature of bacterial pathogens. Research is required to provide a greater understanding of the ecology and epidemiology of pathogens such as *E. coli* O157 on farms. The key issue for risk management in the abattoir is not technology, but the operation of some shared cost (primary producers, processors, retailers & consumers) framework so that currently available decontamination technologies can be applied and will no longer remain prohibitively expensive.

REFERENCES

1. Avens J.S., Clayton P., Jones D.K., Bolin R., Lloyd W., Jankow D., Acetic acid spray ineffective on beef carcasses with low bacteria counts. Lebens. Wissen.-Technol., 1996, 29, 28-32.
2. Bacon R.T., Belk K.E., Sofos J.N., Clayton R.P., Reagan J.O., Smith G.C., Microbial populations on animal hides and beef carcasses at different stages of slaughter in plants enjoying multiple-sequential interventions for decontamination. J. Food Prot., 2000, 63, 1080-1086.
3. Barkocy-Gallagher G.A., Arthur T.M., Rivera-Betancourt M., Nou X., Shackelford S.D., Wheeler T.L., Koohmaraie M., Seasonal prevalence of Shiga toxin-producing *Escherichia coli*, including O157,H7 and non-O157 serotypes, and *Salmonella* in commercial beef processing plants. J. Food Prot., 2003, 66, 1978--1986.
4. Bell R.G., Distribution and sources of microbial contamination on beef carcasses. J. Appl. Microbiol., 1997, 82, 292-300.
5. Bolton D.J., Byrne C.M., Sheridan J.J., McDowell D.A., Blair I.S., The survival characteristics of a non-toxigenic strain of *Escherichia coli* 0157,H7. J. Appl. Microbiol., 1999, 86, 407-411.
6. Bolton D.J., Doherty A.M., Sheridan J.J., Beef HACCP, Intervention and non-intervention systems. Int. J. Food Microbiol., 2001, 66, 119–129.
7. Bosilevac J.M., Arthur T.M., Wheeler T.L., Shackelford S.D., Rossman M., Reagan J.O., Koohmaraie M., Prevalence of *Escherichia coli* O157 and levels of aerobic bacteria and *Enterobacteriaceae* are reduced when hides are washed and treated with cetylpyridinium chloride at a commercial beef processing plant. J. Food Prot., 2004, 67, 646-650.
8. Bosilevac J.M., Nou X., Osborn M.S., Allen D.M., Koohmaraie M., Development and evaluation of an on-line hide decontamination procedure for use in a commercial beef processing plant. J. Food Prot., 2005, 68, 265-272.
9. Buchko S.J., Holley R.A., Buchko S.J., Olson W.O., Gannon V.P.J., Veira D.M., The effect of different gain diets on fecal shedding of *Escherichia coli* O157,H7 by steers. J. Food Prot., 2000, 63, 1467-1474.
10. Byrne C.M., Bolton D.J., Sheridan J.J., McDowell D.A., Blair I.S., The effects of pre-slaughter washing on the reduction of *Escherichia coli* O157,H7 transfer from cattle hides to carcasses during slaughter. Lett. Appl. Microbiol., 2000, 30, 142-145.
11. Callaway T.R., Elder R.O., Keen J.E., Anderson R.C., Nisbet D.J., Forage feeding to reduce preharvest Escherichia coli populations in cattle, a review. J. Dairy Sci., 2003. 86, 852-860.
12. Castillo A., Lucia L.M., Goodson K.J., Savell J.W., Acuff G.R., Comparison of water wash, trimming, and combined hot water

and lactic acid treatments for reducing bacteria of faecal origin on beef carcasses. J. Food Prot., 1998, 61.823-828.
13. Chapman P.A., Wright D.J., Higgins,R., Untreated milk as a source of verotoxigenic *Escherichai coli* O157. Vet. Rec., 1993, 133, 171-172.
14. Cray Jr. W.C., Casey T.A., Bosworth B.T., Rasmussen M.A., Effect of dietary stress on fecal shedding of *Escherichia coli* O157,H7 in calves. Appl. Environ. Microbiol., 1998, 64, 1975-1979.
15. Diez-Gonzalez F., Callaway T.R., Kizoulis M.G., Russel J.B., Grain feeding and the dissemination of acid-resistant *Escherichia coli* from cattle. Science, 1998, 281, 1666-1668.
16. Dorsa W.J., Cutter C.N., Siragusa G.R., Effectiveness of a steam-vacuum sanitizer for reducing *Escherichia coli* O157,H7 inoculated to beef carcass surface tissue. Lett. Appl. Microbiol., 1996a, 23 (1), 61-63.
17. Dorsa W.J., Cutter C.N., Siragusa G.R., Koohmaraie M., Microbial decontamination of beef and sheep carcasses by steam, hot water spray washes, and a steam vacuum sanitizer. J. Food Prot., 1996b, 59, 127-135.
18. Dorsa W.J., Cutter C.N., Siragusa G.R., Effects of steam-vacuuming and hot water spray wash on the microflora of refrigerated beef carcass surface tissue inoculated with *Escherichia coli* O157,H7, *Listeria innocua*, and *Clostridium sporogenes*. J. Food Prot., 1997, 60, 114-119.
19. Elder R.O., Keen J.E., Siragusa G.R., Barkocy-Gallagher G.A., Koohmaraie M., Laegreid W.W., Correlation of enterohaemorrhagic *Escherichia coli* O157 prevalence in faeces, hides, and carcasses of beef cattle during processing. Pro. Nat. Acad. Sci., USA., 2000, 97 2999-3003.
20. Gill C.O., Bryant J., Decontamination of carcasses by vacuum-hot water cleaning and steam pasteurizing during routine operations at a beef packing plant. Meat Sci., 1997, 47, 267-276.
21. Gill C.O., Bryant J., Bedard D., The effects of hot water pasteurizing treatments on the appearances and microbiological conditions of beef carcass sides. Food Microbiol., 1990, 16, 281-289.
22. Hancock D.D., Besser T.E., Kinsel M.L., Tarr P.I., Rice D.H., Paros M.G., The prevalence of *Escherichia coli* O157,H7 in dairy and beef cattle in Washington State. Epidemiol. Infect., 1994, 133, 199-207.
23. Hancock D.D., Besser T.E., Rice D.H., Herriot D.E., Tarr P.I., A longitudinal study of *Escherichia coli* O157 in fourteen cattle herds. Epidemiol. Infect., 1997, 118, 193-195.
24. Hancock D., Besser T., Lejeune J., Davies M., Rice D., The control of VTEC in the animal reservoir. Int. J. Food Microbiol., 2001, 66, 71-78.
25. Herriott D.E., Hancock D.D., Ebel E.D., Carpenter L.V., Rice D.H., Besser T.E., Association of herd management factors with colonization of dairy cattle by Shiga toxin-positive *Escherichia coli* O157. J. Food Prot., 1998, 61.802-807.
26. Jordan D., McEwen S.A., Lammerding A.M., McNab W.B., Wilson J.B., Pre-slaughter control of *Escherichia coli* O157 in beef cattle, a simulation study. Previews Vet. Med., 1999, 41, 55–74.
27. Kudva I.T., Hunt C.W., Williams C.J., Nance U.M., Hovde C.J., Evaluation of dietary influences on *Escherichia coli* O157,H7 shedding by sheep. Appl. Environ. Microbiol., 1997, 63, 3878--3886.
28. Kudva I.T., Blanch K., Hovde C.J., Analysis of *Escherichia coli* O157,H7 survival in ovine or bovine manure and manure slurry. Appl. Environ. Microbiol., 1998, 64, 3166-3174.
29. Lynn T.V., Hancock,D.D., Besser T.E., Harrison J H., Rice D.H., Stewart N.T., Rowan L.L., The occurrence and replication of *Escherichia coli* in cattle feeds. J. Dairy Sci., 1998, 81, 1102-1108
30. Maule A., Survival of verocytotoxigenic *Escherichia coli* O157 in soil, water and on surfaces. Symposium Series for Society of Applied Microbiology, 2000, 29, 71S-78S.
31. Nesbakken T., Nerbrink E., Røtterud O.J., Borch E. Reduction of *Yersinia enterocolitica* and *Listeria* spp. on pig carcasses by enclosure of the rectum during slaughter. Int. J. Food Microbiol., 1994, 23, 197-208.
32. Nielsen E.V., Tegmeier C., Andersen H.J., Gronbaek C., Andersen J.S., Influence of age, sex and herd characteristics on the occurrence of verocytotoxin-producing *Escherichia coli* O157 in Danish dairy farms. Vet. Microbiol., 2002, 88, 245-257.
33. Nisbet D.J., Corrier D.E., DeLoach J.R., Effect of mixed cecal microflora maintained in continuous culture, and dietary lactose on Salmonella typhimurium colonization in broiler chicks. Avian Dis., 1993, 37, 528–535.
34. Nou X., Rivera-Betancourt M., Bosilevac J.M., Wheeler T.L., Shackelford S.D., Gwartney B.L., Reagan J.O., Koohmaraie M., Effect of chemical dehairing on the prevalence of *Escherichia coli* O157,H7 and the levels of aerobic bacteria and Enterobacteriaceae on carcasses in a commercial beef processing plant. J. Food Prot., 2003, 66, 2005-9
35. Nisbet D.J., Corrier D.E., DeLoach J.R., Effect of mixed cecal microflora maintained in continuous culture, and dietary lactose on Salmonella typhimurium colonization in broiler chicks. Avian Dis., 1993, 37, 528–535.
36. Nutsch A.L., Randall K., Phebus R.K., Riemann M.J., Kotrola J.S., Craig Wilson R., Boyer J.E., Brown T.L., Steam pasteurization of commercially slaughtered beef carcasses, evaluation of bacterial populations at five anatomical locations. J. Food Prot., 1998, 61, 571-577.
37. Prasai R.K., Acuff G.R., Lucia L.M., Hale D.S., Savell J.W., Morgan J.B., Microbiological effects of acid decontamination of beef carcasses at various locations in processing. J. Food Prot., 1991, 54, 868-872.
38. Rivera Betancourt M., Shackelford S.D., Arthur T.M., Westmoreland K.E., Bellinger G., Rossman M., Reagan J.O., Koohmaraie M., Prevalence of *Escherichia coli* O157,H7, *Listeria monocytogenes*, and *Salmonella* in two geographically distant commercial beef processing plants in the United States. J. Food Prot., 2004, 67, 295-302.
39. Russell J.B., Diez-Gonzalez F., Jarvis G.N., Effects of diet shifts on *Escherichia coli* in cattle. J. Dairy Sci., 2000, 83, 863-873.
40. Sargeant J.M., Sanderson M.W., Smith R.A., Griffin D.D., Associations between management, climate and *Escherichia coli* O157 in the faeces of feedlot cattle in the Midwestern USA. Pre. Vet. Med., 2004, 66, 175-206.
41. Schnell T.D., Sofos J.N., Littlefield V.G., Morgan J.B., Gorman B.M., Clayton R.P., Smith G.C., Effects of post-exsanguination dehairing on the microbial load and visual cleanliness of beef carcasses. J. Food Prot., 1995, 58, 1297-1302.
42. Sheridan J.J., Sources of contamination during slaughter and measures for control. 1998, *in*: Food Safety The Implications of Change From Producerism to Consumerism (eds. J.J.Sheridan, M. O'Keeffe, M. Rogers). Food & Nutrition Press, Inc., USA, pp. 137-155.
43. Small A., Reid C.-A., Avery S., Buncic S., Potential for the spread

of food-borne pathogens in the cattle lairage environment. 2000, *in*: Proceedings of the first Euroconference on food safety assurance and veterinary public health, Event 1 food safety assurance in the pre-harvest phase. Vienna, Austria, p. 86.

44. Snijders J.D., Wells J.G., Yashuk J., Puhr N., Blake P.A., Outbreak of invasive *Escherichia coli* on a cruise ship. American J. Trop. Med. Hyg., 1984, 32, 281-284.
45. United States Department of Agriculture (USDA), Food Safety Inspection Service (FSIS). 2000. Risk assessment for *E. coli* O157,H7 in ground beef.
46. Van Donkersgoed J., Jericho K.W.F., Grogan H., Thorlakson B., Pre-slaughter hide status of cattle and the microbiology of carcasses. J. Food Prot., 1997, 60, 1502–1508.
47. Wang G., Zhao T., Doyle M.P., Fate of enterohemorrhagic *Escherichia coli* O157,H7 in bovine faeces. Appl. Environ. Microbiol., 1996, 62, 2567-2570.
48. Zhao T., Doyle M.P., Harmon B.G., Brown C.A., Mueller P.O., Parks A.H.,. Reduction of carriage of enterohemorrhagic *Escherichia coli* O157,H7 in cattle by inoculation with probiotic bacteria. J. Clin. Microbiol., 1998, 363, 641–647.