Characterization of Vancomycin-Resistant *Enterococcus faecium* Isolates from Broiler Poultry and Pig Farms in England and Wales

L. Garcia-Migura, E. Pleydell, S. Barnes, R. H. Davies, and E. Liebana*

Department of Food and Environmental Safety, Veterinary Laboratories Agency-Weybridge, Addlestone, Surrey KT15 3NB, United Kingdom

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This study aimed to investigate the occurrence and molecular epidemiology of vancomycin-resistant *Enterococcus faecium* (VREF) isolates on poultry and pig farms in England and Wales. A total of 217 VREF isolates were obtained from fresh feces and environmental swabs collected from conventional and organic farms. A predominant pulsed-field gel electrophoresis (PFGE) profile was found for each VREF-positive farm, together with less frequent types. All isolates presented the *vanA* genotype and were *esp* negative. Seventy-six percent of the VREF isolates were additionally resistant to nine or more antimicrobials, presenting a diverse range of resistance phenotypes. The multiresistance traits did not appear to be specific to individual farms or sample types (i.e., environmental or fecal), nor did they correlate with any specific PFGE type. Ninety-three percent of the isolates were resistant to penicillin, 89% were resistant to tetracycline, 87.5% were resistant to erythromycin, and 50% were resistant to quinupristin-dalfopristin (Synercid). The lack of clonality among these populations may suggest the horizontal transfer of resistance genes and/or a dynamic replacement of clonal lines rather than persistence.

Bacteria are capable of developing strategies to enhance their survival in adverse situations such as conditions of high salinity, changes in temperature, the presence of heavy metals, or the presence of antimicrobial activity. *Enterococcus faecium*, which is part of the normal intestinal flora in humans and animals (12), provides a good example of such adaptive bacterial evolution. This species has emerged as an important opportunistic pathogen causing life-threatening infections in hospitals. The emergence of this pathogen is associated with a remarkable capacity to accumulate resistance to antimicrobials (13). Enterococci are intrinsically resistant to cephalosporins and aminoglycosides and were the first bacteria to acquire resistance to vancomycin (19), a glycopeptide antibiotic commonly used for the clinical therapy of gram-positive nosocomial infections.

Multidrug-resistant enterococci, particularly those that are vancomycin resistant, are a major cause of concern for the medical community. The percentage of infections caused by vancomycin-resistant enterococci (VRE) has increased 20-fold over the last decade in the United States (13). Recently, it was shown that the genes responsible for this resistance have the potential to be transferred to other gram-positive pathogens such as *Staphylococcus aureus* (20), thus intensifying the public health threat, especially now that the first cases of vancomycin- and methicillin-resistant *S. aureus* have been reported in the United States (26, 30).

Antimicrobial growth promoters have been used for the past five decades as an effective way of enhancing productivity and animal health during livestock production. Avoparcin is an example of a feed additive that has been used in intensive and integrated agricultural systems across Europe, especially within the pig and poultry industries. This growth promoter has not been used in the United States.

Avoparcin is a glycopeptide that induces cross-resistance to vancomycin (32) and has been considered an important factor in the emergence and spread of resistance to vancomycin in enterococcal populations (4). For this reason, the use of avoparcin was banned in Denmark in 1995 and throughout the European Union in 1997.

In Europe, VRE carriage occurs mainly among healthy individuals in the community and in farm livestock (8). In contrast, in the United States VRE are normally endemic to hospitals (8, 10, 15). VRE have also recently been found as a result of infections linked to foreign travel and to the consumption of imported food (17). It has been postulated that in Europe, the colonization of humans by VRE may occur predominantly via the food chain (5, 6). Moreover, similar *vanA* elements have been found in strains from animals and humans (9, 25, 29). Although the colonization of humans by animal isolates might be “transient” (7, 24), the risk of transfer of resistance genes during the colonization period has not yet been assessed and might be crucial.

Multiresistant organisms might enter the food chain via farm animals. The aim of the present study was to investigate the occurrence and molecular epidemiology of vancomycin-resistant *E. faecium* (VREF) on poultry and pig farms in England and Wales. The molecular characterization of isolates collected from farms (including genotyping and identification of specific resistance determinants) will help to develop a better...

* Corresponding author. Mailing address: Department of Food and Environmental Safety, Veterinary Laboratories Agency-Weybridge, Addlestone, Surrey KT15 3NB, United Kingdom. Phone: 44 1932 357587. Fax: 44 1932 357595. E-mail: e.liebana@vla.defra.gsi.gov.uk.
understanding of the persistence and dynamics of these organisms in farm environments.

MATERIALS AND METHODS

Farms and sample collection. Samples were collected from a total of 47 farms. Twenty-five farms (six conventional and seven organic broiler farms and seven conventional and five organic pig farms) were sampled once in 2002 and then again within the same year in 2003. A small number of farms were visited more frequently in order to make preliminary assessments of short-term variability.

During each visit, 40 to 60 pooled fecal samples were collected, ensuring that all age groups or production classes present on each farm were represented. Pooled fecal samples were collected from the ground and consisted of approximately 5 g of fecal material from each of eight fresh fecal masses. Care was taken during the procedure to avoid environmental contamination of the fecal samples.

Representative environmental swabs were also taken from internal building surfaces, feeders, and drinkers. For the remaining 22 farms, the farmers collected eight pooled fecal samples from three of their livestock houses on a single occasion and sent them by post to the laboratory for analysis.

Bacteriology. Fecal samples (40 g) were mixed with the same volume of phosphate-buffered saline and vortexed, and then 10 μl of each was streaked onto plates of Brain Heart Agar (Oxoid, Basingstoke, United Kingdom) containing vancomycin (6 μg/ml) and incubated aerobically for 48 h at 42°C. Environmental samples were enriched in buffered peptone water for 18 h at 37°C before plating onto the same selective medium. Presumptive VREF isolates were selected by their colony morphology and color (purple-pink colonies with a lighter halo), subcultured, and stored at −70°C. The identity of the presumptive E. faecium isolate was confirmed by real-time PCR. Amplification was performed in a LightCycler instrument (Roche Diagnostics UK Ltd., Lewes, United Kingdom) in glass capillaries containing 20-μl reaction mixtures including 1× LightCycler FastStart DNA Master SYBR green I (Roche Molecular Biochemicals), 10 pmol of each of the ddeE-fecA-specific primers F1 and F2 (11), 4 mM MgCl₂ (final concentration), and 2 μl of a crude extract of bacterial DNA template. The PCR run comprised 30 cycles with denaturation at 95°C for 5 seconds, annealing at 50°C for 25 seconds, and extension at 72°C for 22 seconds. The nature of the amplicon was determined by a melting point analysis over a temperature range from 65 to 95°C, with a transition rate of 0.1°C/s and continuous detection of fluorescence in channel 1. The melting temperature (Tm) for the PCR product was 84°C, and the size of the product was 550 bp.

The identification of the genes responsible for vancomycin resistance was investigated by a multiplex PCR as described previously (11). Subsequently, a duplex, SYBR green I-based PCR assay was developed for the simultaneous detection of E. faecium and vanA genes by the use of the primers described above in a LightCycler assay. The PCR consisted of 30 cycles of 95°C for 5 seconds (denaturation), 54°C for 5 seconds (annealing), and 72°C for 25 seconds (extension). A melting point analysis over a temperature range from 65 to 95°C with a transition rate of 0.1°C/s resulted in the identification of two distinct peaks representing the two targets, i.e., one at 84°C for ddeE-fecA and the other at 87°C for vanA.

The presence of the esp gene in 137 randomly selected isolates was assessed by PCR. Primers were designed with DnaStar software from a published sequence of the esp gene of E. faecium (GenBank accession number AYX537383). PCRs were prepared as follows: 25-μl volumes contained 10 pmol of each primer (esp1, 5’TATGGCGGAAACAGGCTCAG; esp2, 5’TTGTTGACATTTTCA TAGC). 1.5 mM MgCl₂, 1 U of AmpliTaq Gold (Applied Biosystems), 1× GenAmp PCR buffer, and 5 μl of DNA template. The PCR cycling parameters were as follows: denaturation at 94°C for 10 min, followed by 30 cycles consisting of 94°C for 30 seconds, 62°C for 1 min, and 72°C for 3 min and a final cycle of 10 min at 72°C. The expected size of the amplicon was 471 bp.

PFGE. DNA was prepared as described previously (9a). A single colony of each isolate was streaked onto yeast extract agar and incubated overnight at 37°C. Using a cotton swab, we transferred a portion of the growth to 3 ml of TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) and adjusted the cell concentration to 0.59 with a Dade Microscan turbidity meter (Dade Behring). A total volume of 240 μl of the suspension was transferred to 1.5-ml microcentrifuge tubes, and 60 μl of lysosome solution (10 mg/ml) was added. The tubes were incubated at 37°C for 10 min. Immediately after incubation, 300 μl of 1.2% Seakem Gold agarose (Cambrex, East Rutherford, N.J.)–1% sodium dodecyl sulfate–0.2 mg/ml proteinase K was mixed with the bacterial suspension K and pipetted into disposable plug moulds. Three plugs per isolate were transferred to 50-ml polypropylene screw-top tubes with 5 ml of cell lysis buffer (50 mM Tris, 50 mM EDTA, pH 8.0, 1% sarcosyl, 0.15 mg/ml proteinase K) and then incubated at 54°C in a shaking water bath for 2 h. Thereafter, the plugs were washed twice with 15 ml of sterile water and four times with TE buffer at 50°C for 15 min. Restriction digestion of chromosomal DNAs was carried out by using 25 units of Smal (Promega, Southampton, United Kingdom) for 2 h at 25°C. Pulsed-field gel electrophoresis (PFGE) was performed on a CHEF DR III system (Bio-Rad, Hercules, Calif.) in 0.5× TBE extended-range buffer (Bio-Rad) with recirculation at 14°C. DNA restriction fragments were resolved in 0.8% SeaKem Gold agarose in 0.5× TBE buffer. DNA from Salmonella Braenderup H9812 restricted with XbaI was used as a size marker. Restriction fragments were resolved under the running conditions described by Turabelidze et al. (28). Macromutation patterns were compared by the use of BioNumerics software (Applied Maths, Sint-Martens-Latem, Belgium).

Antimicrobial susceptibility testing. Resistance patterns and MICs were ascertained for 16 different therapeutic and growth-promoting antimicrobials by a standard Susceptibility protocol (Trek Diagnostic Systems Ltd., England). Table 1 gives the concentrations of antimicrobials tested and the breakpoint used for each drug (DANMAP 2002). Briefly, isolates were grown in yeast extract agar. Subsequently, a 0.5 McFarland cell suspension was prepared in demineralized water, and 10 μl was inoculated into 10 ml of cation-adjusted Mueller-Hinton broth (Trek Diagnostic Systems, Ohio) for a final inoculum of 10°C CFU/ml. Aliquots of 50 μl of the inoculum were seeded in each well of a microtiter plate, which contained doubling dilutions of the antimicrobials. The plates were sealed and incubated aerobically at 37°C for 24 h. A growth control well was used as a reference for interpreting the growth patterns in each plate. Pseudomonas aeruginosa (ATCC 27853), Enterococcus faecalis (ATCC 29213), S. aureus (ATCC 29213), and Echerichia coli (ATCC 25922 and ATCC 35218) were used as quality control organisms. The MIC was recorded as the lowest concentration of antimicrobial that inhibited visible growth.

RESULTS

A total of 217 VREF isolates were obtained from fecal and environmental samples during the study period (January 2002 to February 2003).

When comparing the sensitivities of the two different sampling techniques (sampling by farmers versus sampling by the research team) for the detection of VREF by using the unpaired t test, we found no evidence to reject the null hypothesis that there was no difference between the two collection methods (P = 0.457).

Broiler farms. Twenty-seven of the 33 farms investigated were found to have at least one VREF-positive sample on at least one occasion over the course of the study. All 20 conventional broiler farms that submitted pooled fecal samples by post were found to be positive for VREF. The number of fecal samples from these farms which contained VREF ranged from...
one of eight to eight of eight. Of the further 13 farms that were
visited in person, 35 to 100% of fecal samples from three of the
six conventional broiler farms were found to be positive for
VREF on every visit. Environmental swabs taken on these
farms were also positive. On one conventional farm, VREF
isolates were found in environmental swabs collected from
the surfaces of two houses that had been cleaned and disinfected,
albeit the organisms had not been found in fecal samples
collected from the previous flock. Fecal VREF isolates were
also found on three visits to two organic farms, but at a lower
prevalence than that for the conventional farms (3 to 10% of
fecal samples). On another organic farm, VREF isolates were
found in environmental samples, but not in fecal samples, on
one of five visits.

Pig farms. Fewer VREF isolates were isolated from pig
farms in this study, although 4 of the 14 farms investigated did
yield at least one positive sample. The two conventional fin-
isher units that sent pooled fecal samples to the laboratory
were found to have one of eight and five of eight positive
samples. Fecal samples from 2 of the 12 farms that were sam-
pred more intensively were positive for VREF at low levels (2
to 5%) on a single visit. One of these farms was conventional
and the other was organic. We failed to find any VREF on a
second visit to every one of those 12 farms.

Genotyping. All VREF isolates harbored the vanA resist-
ance gene and all selected isolates were exp negative. They all
exhibited MICs of >64 µg/ml of vancomycin and between 8
µg/ml and ≤64 µg/ml of teicoplanin. One hundred nineteen
PFGE restriction profiles were identified among the 217 iso-
lates by Smal-PFGE (Fig. 1). Each profile was given a unique
identification number. The number of fragments generated
ranged from 16 to 24, and their sizes varied from 20 to 350 kb.
Our analysis revealed a predominant PFGE profile for each
farm together with less represented types. The most prevalent
type from one visit was never detected again in isolates from a
second visit.

Two isolates from farms 3 and 8 were highly related accord-
ing to PFGE. In order to check the stability of the PFGE types,
we repeatedly passaged one colony of each of two presump-
tively related isolates in the laboratory. PFGE was then re-
peated with the new subcultures. During the third passage, a
new band appeared in the PFGE profile of one of the isolates.
This suggests that small changes in PFGE profiles may occur in
short periods of time.

Antimicrobial resistance. Ninety-three percent of the VREF
isolates were resistant to penicillin (MIC, ≥16 µg/ml), 89%
were resistant to tetracycline (MIC, ≥16 µg/ml), 88% were
resistant to erythromycin (MIC, ≥8 µg/ml), 79% were resistant
to streptomycin (MIC, ≥2,048 µg/ml), and 50% were resistant
to quinupristin-dalfopristin (Synercid) (MIC, 4 to 32 µg/ml).
One isolate displayed resistance to chloramphenicol, with an
MIC of 32 µg/ml, and 8% of the isolates were found to be of
intermediate resistance (MIC, 16 µg/ml). None of the strains
exhibited high-level resistance to gentamicin, and all were sen-
sitive to florfenicol (MIC, ≥32 µg/ml). With the exception of
vancomycin and teicoplanin, no correlation among resistances
to several antimicrobials was found in this study.

An analysis of the resistance profiles indicated that 166
(76%) of the 217 VREF isolates tested were resistant to nine
or more antimicrobials, comprising a diverse range of pheno-
types (n = 70). Eleven percent of the isolates were resistant to
the same 12 antimicrobials, as follows: bacitracin (MIC, ≥256
µg/ml), ciprofloxacin (MIC, 4 µg/ml), erythromycin (MIC, >32
µg/ml), flavomycin (MIC, ≥32 µg/ml), kanamycin (MIC,
≥2,048 µg/ml), nitrofurantoin (MIC, 128 to 256 µg/ml), pen-
icillin (MIC, 16 to 128 µg/ml), streptomycin (MIC, ≥2,048
µg/ml), quinupristin-dalfopristin (MIC, 4 to 16 µg/ml), tetra-
cycline (MIC, >32 µg/ml), vancomycin (MIC, >64 µg/ml), and
teicoplanin (MIC, ≥64 µg/ml). These 24 isolates were all ob-
tained from different conventional poultry farms. Finally, 23%
of the isolates were resistant to 11 antimicrobials, 25% were
resistant to 10, 17% were resistant to 9, and 11.4% were resis-
tant to 8.

Table 2 shows the distribution of genotypes and phenotypes
on farms with more than one VREF isolate.

DISCUSSION

VREF was found in samples from 31 of 47 farms (66%).
However, we identified differences in the percentages of pos-
itive pooled fecal samples between pig and poultry farms. Fur-
thermore, VREF isolates were repeatedly isolated with a rel-
atively high sample prevalence from conventional broiler
farms. Far fewer samples from organic poultry yielded VREF,
and VREF was never isolated from any organic broiler farm on
more than one occasion. In the United Kingdom, certified
organic farms must agree to relatively restrictive policies
in terms of antimicrobial usage (http://www.defra.gov.uk/farm/or-
ganic/legislation-standards). For instance, organic farms are
not permitted to use antimicrobial growth-promoting agents,
but they may administer therapeutic treatment if this is judged
to be clinically necessary by a veterinary surgeon. None of the
participating organic poultry farms used any antimicrobials
during this study, and most of them (6/7) had not used such
treatment within the 12 months preceding the study.

On one conventional farm, VREF was detected in samples
originating from floors, walls, wooden roof supports, and dust
taken after cleaning and disinfection. This indicates that insuf-
cient cleaning and disinfection may play a role in the persis-
tence of these organisms. Interestingly, all isolates from this
farm (which used no therapeutic antimicrobials but did use in-feed avilamycin) were of a single clone. The same pattern
was also detected in a positive nondomestic bird sample col-
lected from the concrete concourse outside the houses and in
dust samples from an occupied house collected during the
same visit. Suitable routine hygiene protocols should be per-
formed thoroughly to prevent the persistence of these organ-
isms on surfaces and to minimize colonization by resistant
strains when new flocks enter the premises.

Multidrug-resistant VREF has been described previously (1,
2, 14), but the level of multiresistance among isolates found in
the present study appears to be unprecedented. To date, such
multiresistant profiles have not been reported elsewhere in
Europe for animal sources. It is difficult to evaluate the signif-
icance of these differences since the use of antimicrobials is
a common practice in most European countries. It is also diffi-
cult to assess the significance of potential horizontal gene
transfer between other bacteria and E. faecium clones in our
study, and this would require a detailed characterization of the
different genetic elements involved.
From the medical point of view, the emergence of multiresistance among VREF isolates is a great cause for concern, since some of the antimicrobials involved are commonly used for the treatment of human VRE infections. This is well illustrated by the examples of gentamicin and streptomycin, which have a synergistic effect when administered with cell wall inhibitors such as vancomycin (18). A high-level resistance to aminoglycosides might pose a serious risk in hospitals, as antimicrobial therapy could be limited. In this study, while no isolates were resistant to high levels of gentamicin, 70% were resistant to high levels of streptomycin. A combination of streptogramins (quinupristin-dalfospristin [Synercid]) has been successfully used for the treatment of VREF infections (27). However, some E. faecium strains have already acquired resistance to these antibiotics. This resistance may be related in part to the use of virginiamycin, a growth-promoting feed additive incorporated in agriculture for poultry and pig production (32). The use of virginiamycin was banned in Denmark in 1998 and in the rest of the European Union in 1999. In the present study, 50% of the isolates were resistant to quinupristin-dalfospristin. Erythromycin resistance was also found at very high levels on every farm. Macrolide resistance encoded by \textit{ermB}-type genes has been linked to the same conjugative plasmid harboring \textit{vanA} genes (3). Further studies are being carried out to ascertain the possible role of gene linkage and the coselection of VREF on farms in the United Kingdom as a result of the use of macrolides.

The multiresistance profiles found in this study do not seem to be specific to a particular farm or sample type, and they do not correlate with any specific PFGE banding pattern.

### TABLE 2. Distribution of dominant phenotypes and genotypes on farms

<table>
<thead>
<tr>
<th>Farm no. (date of study)</th>
<th>No. of VREF isolates</th>
<th>No. of phenotypes</th>
<th>Dominant phenotype(s)(^a)</th>
<th>% of isolates belonging to dominant type</th>
<th>No. of genotypes</th>
<th>Dominant genotype(s)</th>
<th>% of isolates belonging to dominant type</th>
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<td>2</td>
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<td>50</td>
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<td>100</td>
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<td>71</td>
<td>50</td>
</tr>
</tbody>
</table>

\(\text{\textsuperscript{a}}\) Bc, bacitracin; Cp, ciprofloxacin; Ey, erythromycin; Fa, flavomycin; Kn, kanamycin; Nt, nitrofurantoin; Pn, penicillin; Sr, streptomycin; Sn, synercid; Tr, tetracycline; Vn, vancomycin; Ti, teicoplanin.

\(\text{\textsuperscript{b}}\) Organic farm.

\(\text{\textsuperscript{c}}\) Pig farm.

\(\text{FIG. 1. Dendrogram generated by Gel Compar II software showing the relationships of 119 representative fingerprints (SmaI-PFGE types) for 217 VREF isolates. The analysis of the generated bands was performed by using the Dice coefficient and the unweighted-pair group method with arithmetic averages (optimization of 0.00\% and position tolerance of 1.00\%).}\)
though PFGE is still considered the standard typing method for enterococci, there are no standardized criteria for analyzing PFGE patterns (16). Therefore, the interpretation of the results can lead to different conclusions. In addition, the lack of standardization in terms of PFGE running conditions makes interlaboratory comparisons rather limited. The issue of how many band differences account for the description of a new clone has not yet been resolved. This is especially true for long-term studies. In the present study, there was only one case of related PFGE types being isolated on two different premises that were spatially separated and managed by different farmers. Farm 8 was a conventional poultry farm which supplied stock to farm 3 (organic poultry farm). Interestingly, similar PFGE profiles were found on each of these farms, differing in only one band. The link between farms was curtailed during the course of the study, and subsequent visits to farm 3 did not recover further VREF isolates. This event might suggest a transfer of resistant strains from farm to farm.

PFGE has provided evidence of the high level of genetic diversity among E. faecium populations in farm environments in the United Kingdom. The PFGE results of our study suggest that the introduction of new clones to the farm by deliveries of new stock or by other reservoirs (e.g., other domestic and wild animals, feed, litter, and water), rather than the persistence of resistant clones, may be the cause for the persistence of vancomycin resistance on these farms. Alternatively, we may have observed the effects of the dynamic interaction of bacterial populations, by which the previously detected clone called “the dominant type” may still be present on the farm on subsequent visits, but at levels below the limit of detection, whereas “new” clones that previously survived in small numbers may increase to detectable levels for unknown reasons. Management practices such as the use of disinfectants, sources of replacement stock, or even interactions with other enteric flora might have an important impact on the selection of the new dominant bacterial population.

VREF isolates from different habitats are very polyclonal, suggesting the horizontal gene transfer of the vancomycin resistance genes rather than the spread of a single clone. Therefore, if we cannot find the same clones in different environments, but we are able to find the same resistance genes, some clear questions arise. Where is the transfer of van and other resistance genes occurring? Are farm animals a significant long-term reservoir of these resistance genes, and if so, since avoparcin has not been administered to livestock since 1999, are we coselecting for vancomycin resistance by the use of other compounds? There may also be other undetermined factors indirectly selecting for the persistence of the vancomycin resistance genes if these organisms are more suited for environmental survival.

The esp gene encodes an enterococcal surface protein (Esp), which contributes to the colonization and infection of the urinary tract by increasing attachment to epithelial surfaces and biofilm production (22). This gene appears to be an enterococcus-specific virulence factor which is highly conserved in E. faecium subpopulations involved in hospital outbreaks (31), independent of the vancomycin susceptibility (23). The isolates tested in our study were negative for this element, which suggests that farm animals may not be a significant source of these genes. The absence of esp genes in our isolates indicates that it is unlikely that they could cause infections in humans, although “in vitro” conjugative transfer of the esp gene has been demonstrated (21).

In our study, we detected antimicrobial resistance even on farms where antimicrobials had not been used for many years, if at all. The factors promoting the persistence of resistant bacteria or resistance genes are not clear. Ideally, farm antimicrobial usage and hospital policies should be implemented to minimize the further development, spread, and persistence of resistant organisms. Statistically representative surveys should be carried out to detect and quantify specific genes in agricultural systems as well as in hospital environments. In addition, epidemiological studies to help to unravel the mechanisms underlying the observed heterogeneity of VREF isolates should be attempted. This would provide useful information to help to prevent and ultimately control the spread of antimicrobial resistance among bacteria.

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REFERENCES


