Distribution of the \textit{ermG} Gene among Bacterial Isolates from Porcine Intestinal Contents

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The \textit{ermG} gene was first found in the soil bacterium \textit{Bacillus sphaericus}. More recently, it was found in several human intestinal \textit{Bacteroides} species. We report here the first finding of \textit{ermG} genes in gram-positive bacteria isolated from porcine feces and from under-barn manure pits used to store porcine wastes. The porcine \textit{ermG} sequences were identical to the sequence of the \textit{B. sphaericus} \textit{ermG} gene except that six of the seven \textit{ermG}-containing strains contained an insertion sequence element insertion in the C-terminal end of the gene. The porcine \textit{ermG} genes were found in three different gram-positive genera, an indication that it is possible that the gene is being spread by horizontal gene transfer. A segment of a \textit{Bacteroides} conjugative transposon that carries an \textit{ermG} gene cross-hybridized with DNA from six of the seven porcine isolates, but the restriction patterns in the porcine strains were different from that of the \textit{Bacteroides} conjugative transposon.

Macrolide antibiotics are used widely to treat human bacterial infections, but they are also used in agriculture. Tylosin is an example of a macrolide antibiotic that is used as a growth promoter and is used to prevent infections in young pigs (20). Previously, we showed that the \textit{ermG} gene, a gene that confers resistance to macrolides, has appeared relatively recently in human colonic \textit{Bacteroides} species (15), where it was found in several species and on two conjugative transposons (CTns) (4, 12, 17). The only previously reported \textit{ermG} gene was found in \textit{Bacillus sphaericus} (11), a soil bacterium and insect pathogen that is being used commercially to control mosquito larvae. The finding of \textit{ermG} genes in human colonic \textit{Bacteroides} species raised the question of how widely the \textit{ermG} gene might be distributed in nature, especially in bacteria that are routinely exposed to macrolides. Since growing and finishing pigs are often fed the macrolide tylosin as a growth promoter and to prevent disease, the intestinal bacteria of pigs seemed a possible source where other examples of \textit{ermG} might be found.

Accordingly, we surveyed 48 pure-culture (PC) isolates obtained from porcine feces or from under-barn manure storage pits at the Fermentation Biotechnology Research Unit, National Center for Agricultural Utilization Research, Peoria, Ill. In this swine production facility, tylosin is included in feed for growing and finishing pigs at a concentration of 10 to 60 g per ton. Cultivation methods were described previously (5). These strains were selected initially as resistant to erythromycin/tylosin or tetracycline. Tetracycline-resistant strains were included in our survey, along with the erythromycin/tylosin-resistant strains, because a preliminary survey had indicated that some of the tetracycline-resistant strains might contain \textit{ermG}.

The 48 isolates were screened by DNA hybridization, using DNA extracted from the resistant strains. The \textit{ermG} probe was the 445-bp internal fragment generated by PCR from the \textit{ermG} gene carried by the \textit{Bacteroides} conjugative transposon CTnGERM1. This gene has more than 99% sequence identity with the \textit{B. sphaericus} \textit{ermG} gene (13). The primers amplified a region of the \textit{Bacteroides} \textit{ermG} gene that extended from bp 265 to bp 710 at the end of the \textit{ermG} gene (15). The sequences of the two primers were as follows: for bp 265, CATTTCTTA GGCACAATCCCAT (forward primer), and for bp 710, TTAA ATAGCGAGAATTTGTCG (reverse primer). Primers were synthesized by Operon Technologies Inc. The conditions used for DNA isolation and for the hybridization screen have been described previously (15). The hybridization analysis identified seven strains that contained \textit{ermG} (Table 1).

To obtain a fuller picture of \textit{erm} and \textit{tet} genes found in the porcine strains that contained \textit{ermG}, we also used as probes internal segments from \textit{ermA}, \textit{ermB}, \textit{ermC}, \textit{ermF}, \textit{ermQ}, and \textit{ermT} as well as the \textit{tet} genes \textit{tetM} and \textit{tetQ}. These probes have been described previously (15, 19). They were chosen because they represent \textit{erm} and \textit{tet} genes that, unlike \textit{ermG}, are known to have a wide distribution in different genera of bacteria. Whereas \textit{ermF} and \textit{tetQ} are prevalent in \textit{Bacteroides} and related genera, \textit{ermA}, \textit{ermB}, and \textit{tetM} have been found in a variety of gram-positive bacteria as well as in some gram-negative bacteria (1, 2, 13). The \textit{ermQ} and \textit{ermT} genes have been found primarily in gram-positive bacteria (3, 19). As shown in Table 1, the \textit{ermA} and \textit{ermC} genes were found in some of the \textit{ermG}-containing strains. The \textit{tetM} gene was found in six of the seven strains. The seventh strain contained an unidentified tetracycline resistance gene because it was phenotypically resistant to tetracycline, but its DNA did not hybridize to any of the \textit{tet} genes probed tested.

When the \textit{erm} probes for genes other than \textit{ermG} were used to screen all 48 of the resistant strains, we found that 2 contained \textit{ermA}, 12 contained \textit{ermB}, 3 contained \textit{ermC}, and 2 contained \textit{ermT}. None of the strains contained the \textit{ermF} gene.
or the \( \text{erm}G \) gene, which has been found primarily in \textit{Clostridium perfringens} (3). Examples of \( \text{tet} \) genes were also found in strains other than those shown in Table 1. Altogether, 3 of the 48 isolates contained \( \text{tet}Q \) and 25 (about 50\%) contained the \( \text{tet}M \) gene. These results show that a number of \( \text{tet} \) and \( \text{erm} \) genes that have been found previously in human clinical isolates are also found in porcine intestinal bacteria and bacteria from the manure pits.

To characterize further the \( \text{erm}G \) genes identified by hybridization, we wanted to compare sequences from these genes with sequences from known \( \text{erm}G \) genes. Initially, crude boiled cell extracts were used as the source of DNA for PCR amplification. Amplicons were sequenced and compared to the sequences of the few known \( \text{erm}G \) genes in the databases. The \( \text{erm}G \) gene selected for amplification was chosen because it covered the region of variability between the \textit{B. sphaericus} and \textit{Bacteroides} genes (15). Also, these primers were found to be the most reliable when used to amplify DNA from the porcine isolates. The PCR survey of DNA from boiled cells identified \( \text{erm}G \) in only four of the isolates shown in Table 1. The sequences of the four PCR amplicons were all identical to the sequence of the \( \text{erm}G \) gene found on a plasmid in \textit{B. sphaericus} (M15332). They differed from the \( \text{erm}G \) genes found in the \textit{Bacteroides} spp. by only one to five nucleotides. The failure of PCR amplification to find the \( \text{erm}G \) genes in the other three strains identified by DNA hybridization was probably due to inhibitors in the boiled cell extracts because PCR products were obtained from the three additional strains when purified DNA was used as the template. The \( \text{erm}G \) sequences of these three additional strains were identical to the \( \text{erm}G \) gene sequences from the other porcine isolates.

An unexpected feature of the PCR products obtained from six of the seven \( \text{erm}G \)-containing strains was that the amplified DNA was predominantly 2.5 kb in size, with a very faint product of the expected 445-bp size. Hybridization analysis indicated that both bands contained \( \text{erm}G \) sequences (data not shown). PC21 was the only strain whose DNA produced only a 445-bp amplicon. The larger product proved to be due to the insertion of a foreign DNA segment that had occurred at bp 671 of the \( \text{erm}G \) sequence, very close to the reverse primer (bp 710 to bp 687 [Fig. 1]). The presence of the 445-bp amplicons in PCRs from all six strains that contained insertions indicated that the insertion was missing in a low percentage of the cells, possibly due to excision or loss of the insertion. A complete sequence of the insertion, named IS PC252, was obtained (AY904360).

There was no significant nucleotide identity between IS PC252 and anything in the databases, but the deduced amino acid sequences of proteins encoded by the two open reading frames (Orfs) (Fig. 1) were similar to those of several gram-negative bacteria.

![FIG. 1. Map of the IS element found integrated in the C-terminal end of \( \text{erm}G \) from six PC isolates. The map is from the sequence of the 2.5-kb PCR amplicon obtained for six of the seven PC strains containing \( \text{erm}G \). The insertion sites in all six strains were identical, and the partial sequences of both ends of the insertion and the \( \text{erm}G \) gene sequences were identical. The \( \text{erm}G \) primers are described in the text. The \( \text{erm}G \) sequences are indicated by the dark arrows and labeled with base pair numbers of the \( \text{erm}G \) sequence from \textit{B. sphaericus}. Sequences of the \( \text{erm}G \) genes can be found under the following GenBank accession numbers: the \( \text{erm}G \) genes from \textit{Bacillus sphaericus} and the PC isolates, M15332; the \( \text{erm}G \) gene from CTnGERM1, AY171301; and the \( \text{erm}G \) gene from CTn7853, L42817. The double-strand sequence of the entire IS element, called IS PC252, was done for PC78A. The two proteins encoded by the open reading frames in the integrated IS are indicated by the arrows. The closest homologs to known genes in the databases are shown below the corresponding arrows for Orf1 and Orf2. They belong to the IS232-like elements found in \textit{Enterococcus faecium} (transposase BAD02264.1 and transposition helper BAD02265.1) and \textit{Bacillus thuringiensis} (IstA and IstB A37801). The E values and the percent sequence identity over percent similarity values are provided. The element is flanked by the 7-bp repeat of bp 665 to 671 of the \( \text{erm}G \) target site.]

### TABLE 1. Characteristics of the seven \( \text{erm}G \)-containing porcine isolates

<table>
<thead>
<tr>
<th>Strain</th>
<th>Site of isolation†</th>
<th>Phenotype (isolated)‡</th>
<th>Genes detected‡</th>
<th>Identification</th>
<th>% Identity†</th>
<th>16S accession no.†</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC3</td>
<td>Pit middle-3′</td>
<td>Tc(^{r}) Em(^{r})</td>
<td>( \text{tet}M, \text{erm}G )</td>
<td>\textit{Clostridium sartagoforme}</td>
<td>99</td>
<td>AF445273</td>
</tr>
<tr>
<td>PC7</td>
<td>Pit middle-3′</td>
<td>Tc(^{r}) Em(^{r})</td>
<td>( \text{tet}M, \text{erm}A, \text{erm}G )</td>
<td>\textit{Clostridium sartagoforme}</td>
<td>100</td>
<td>AF445272</td>
</tr>
<tr>
<td>PC21</td>
<td>Swine feces</td>
<td>Tc(^{r}) Em(^{r})</td>
<td>Unknown ( \text{tet} ) gene, ( \text{erm}G )</td>
<td>\textit{Catenibacterium mitsuokai}</td>
<td>97</td>
<td>AF445225</td>
</tr>
<tr>
<td>PC74</td>
<td>Swine feces</td>
<td>Tc(^{r}) Em(^{r})</td>
<td>Unknown ( \text{tet} ) gene, ( \text{erm}G )</td>
<td>\textit{Clostridium sartagoforme}</td>
<td>99</td>
<td>AF445212</td>
</tr>
<tr>
<td>PC78A</td>
<td>Pit middle-3′</td>
<td>Tc(^{r}) Em(^{r})</td>
<td>( \text{tet}M, \text{erm}G )</td>
<td>\textit{Clostridium sartagoforme}</td>
<td>99</td>
<td>AY52483</td>
</tr>
<tr>
<td>PC81</td>
<td>Pit middle-3′</td>
<td>Tc(^{r}) Em(^{r})</td>
<td>( \text{tet}M, \text{erm}C, \text{erm}G )</td>
<td>\textit{Staphylococcus warneri}</td>
<td>100</td>
<td>AF445277</td>
</tr>
<tr>
<td>PC96B</td>
<td>Pit middle-3′</td>
<td>Tc(^{r}) Em(^{r})</td>
<td>( \text{tet}M, \text{erm}G )</td>
<td>\textit{Clostridium sartagoforme}</td>
<td>99</td>
<td>AF445270</td>
</tr>
</tbody>
</table>

- † The strain designations were assigned to PC isolates from swine feces or swine storage pit manure samples (5).
- ‡ The type of sample from which the isolate was obtained, fecal matter or under-barn manure pit at 3′ (middle) as described by Cotta et al (5).
- § The initial phenotypic isolation, either tetracycline resistant (Tc\(^{r}\)) or tylosine/erythromycin resistant (Em\(^{r}\)), is underlined. All seven of these cultures were both Tc\(^{r}\) and Em\(^{r}\).
- ¶ The genotype or genes were detected by using specific probes and/or by PCR with specific primers (e.g., the \( \text{erm}G \) genes were detected by both methods [this study]).
- † The species and/or genus designations are to the organism with the highest identity according to the 16S rDNA sequence (Fig. 2). The five strains of \textit{Clostridium sartagoforme} differ from each other by one or two nucleotides, and one (PC7) is identical to the partial sequence of \( \text{tet}M \) and \( \text{erm}G \).
- ‡ Percent nucleotide identity over a 445-bp PCR amplicon.
positive insertion sequence (IS) element transposases and transfer helper proteins, e.g., IS232-related elements found in several Bacillus species (7, 9, 10). The insertion resulted in a 7-bp duplication of bp 665 to 671, which flanked the integrated element. Thus, the insertion was probably due to an IS element. A map of the putative IS element, the position of the insertion in the *ermG* genes, and the labeling of the two open reading frames with their nearest homologs in the BLASTP searches are shown in Fig. 1. Orf1 may be truncated or non-functional because the ATG start includes 2 bp of the target sequence and the promoter, if there is one, must originate downstream of the end of the *ermG* gene. The fact that the strains carrying the *ermG* gene that had sustained an insertion were all phenotypically erythromycin resistant indicates that the insertion did not completely inactivate the *ermG* gene; that the IS element could excise, leaving a functional gene; or that other *erm* or erythromycin resistance genes were responsible for the resistance phenotype. If the last alternative is true, there are several isolates (PC3, PC21, PC74, PC78A, and PC96B) that must contain as yet unidentified genes, because no known *erm* gene other than *ermG* was detected by hybridization or PCR in these strains.

Finding identical *ermG* genes, with or without the IS insertion, in the porcine intestinal isolates would suggest that horizontal transfer of the gene has occurred if the gene is found in different genera and species. Accordingly, we determined the 16S rRNA gene sequences from all of the seven porcine isolates that contained *ermG*. The identities of these isolates, as deduced from the rRNA analysis, are shown in Table 1. A dendrogram showing the nearest relatives in the databases is shown in Fig. 2. Previous studies of bacteria in the porcine intestine have shown that gram-positive bacteria predominate (5, 8). As in humans, bacteria of gram-positive genera such as *Clostridium*, *Eubacterium*, and *Lactobacillus* are found in high numbers, although the porcine species are thought to be different from the species that predominate in humans (8, 18, 21, 22). The antibiotic-resistant isolates were virtually all members of these and related genera (Table 1). Four of the five isolates (PC3, PC7, PC78A, and PC96B) that were identified as strains of *Clostridium sartagoforme* were isolated from the middle of the manure pit. The fifth isolate, PC74, was obtained from feces. The 16S rRNA sequence of PC7 was identical to that of *Clostridium sartagoforme*, but the other four strains differed from PC7 and each other by one or two nucleotides and are thus different strains of this *Clostridium* species. In addition, PC7 contained *ermA*. The other two isolates, PC81 (pit) and PC21 (feces), were clearly from different genera. Thus, horizontal transfer of the *ermG* gene between genera seems to have occurred in the case of the porcine isolates. It is interesting that even the form of the *ermG* gene that had sustained an insertion near its 3′ end turned up in strains from species other than *Clostridium sartagoforme*.

Another interesting observation from Fig. 2 is that most of the porcine isolates were related to *Clostridium cocoides*, a species that is found in high numbers in the human colon (22). If animal gram-positive bacterial species are related to these prominent members of the human intestinal bacterial population, transfer of resistance genes from transient animal isolates to these bacteria in the human colon may occur more readily than transfer to numerically predominant gram-negative bacteria, such as *Bacteroides* species. Perhaps the human gram-positive anaerobes, such as *Clostridium cocoides*, should be considered more seriously in the future as possible reservoirs of antibiotic resistance genes.

In *Bacteroides* species, *ermG* is carried on conjugative transposons, such as CTnGERM1 and CTn7853 (12, 17). These CTns are related but are not identical. In a recent study, Wang et al. (17) cloned 7.2 kbp of DNA upstream of the *ermG* gene and 4.5 kbp of DNA downstream of the *ermG* gene. About 3 kb of the upstream clone was virtually identical in DNA sequence to DNA found previously in gram-positive species, such as *Streptococcus pneumoniae* (14). In the streptococci, this 3-kbp region contains a homolog of an ABC transporter of unknown function and *mefA*, a gene that, unlike the *erm* gene, confers resistance to macrolides but not to streptogramins and lincosamides. A 2-kbp region immediately downstream of the *ermG* gene in *Streptococcus* CTnGERM1 contained sequences that had been found previously on a *Staphylococcus aureus ermB*-containing plasmid (6). Other sequences in the downstream region included a homolog of a *Bacteroides fragilis* IS element (17). Hybridization probes consisting of the upstream and downstream regions from CTnGERM1 hybridized to DNA from cells containing CTn7853, although there were restriction site differences (Fig. 3).

To ascertain whether any of the porcine isolates carried DNA that hybridized to the DNA adjacent to *ermG* on CTnGERM1, we used a 7.2-kbp PstI fragment from the region immediately upstream of *ermG* and a 4.5-kbp PstI-AccI fragment downstream of *ermG* as hybridization probes. Neither of these probes contained any *ermG* sequences. The upstream probe hybridized to a 9.4-kbp EcoRV band or a 3.8-kbp HindIII fragment from the six porcine strains with the insertion but not to DNA in PC21 (Fig. 3). The patterns seen for the six PC isolates were all the same, but the patterns were much different and much simpler than those seen in the cases of the *Bacteroides* conjugative transposons CTnGERM1 and CTn7853.
The downstream probe did not hybridize with DNA from any of the PC strains (data not shown). The DNA of PC21 could not be digested with HindIII, indicating that the strain has a HindIII modification system. The DNA was readily digested by EcoRV but did not hybridize to either probe.

Two main conclusions can be drawn from the results presented in this report. First, ermG in antibiotic-resistant isolates from the porcine intestinal tract, all of which were gram-positive bacteria, has been found for the first time. Evidently, ermG is more widely distributed in the bacterial world than appeared to be the case originally. Our results also support the theory that ermG may have originated in the gram-positive bacteria and is being transferred among and between phylogenetically different genera, including the gram-negative bacteria, such as Bacteroides spp.

We have focused on ermG because this might be an indicator of recent gene transfers between gram-positive and gram-negative bacteria and between human and animal intestinal bacteria (13, 15). Yet the results of our study show clearly that the interpretation of such data is not going to be straightforward. Finding genes with high sequence identity in gram-positive and gram-negative bacteria, and in soil, animal, and human bacterial isolates, suggests that promiscuous DNA transfers occur in nature. Ideally, one would also find evidence of a transmissible element carrying the transferred gene. It is intriguing that DNA upstream of the ermG gene in Bacteroides conjugative transposons CTnGERM1 and CTn7853 hybridizes with DNA from six of the seven porcine isolates that carry ermG. However, it is also clear that only a portion of that region is shared. Since we have not cloned and sequenced the upstream and downstream DNA segments that flank ermG in the porcine strains, we cannot be sure that ermG in the porcine isolates is genetically linked to these upstream sequences, as it is in the case of the Bacteroides elements. Indirect support for the hypothesis that the DNA that hybridized to the upstream probe is linked to ermG comes from our finding that DNA in PC81, which is in a different genus than the other five strains, also hybridized to the upstream probe. It is unlikely that the EcoRV and HindIII restriction fragments of the two groups that hybridize to the upstream probe would be the same size unless the region was on an element or part of an element that has been acquired along with the ermG gene. Thus, the cross-hybridizing DNA could be part of a transmissible element that is being transferred among the porcine strains. If so, it is not identical to the ermG-carrying CTns found in Bacteroides species.

**Nucleotide sequence accession numbers.** The sequence of the IS element of PC78A (IS PC252) was submitted to GenBank and given the accession number AY904360. The accession numbers of other sequences deposited in GenBank can be found in Table 1.

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**REFERENCES**


