Erythromycin Resistance and Genetic Elements Carrying Macrolide Efflux Genes in Streptococcus agalactiae

José María Marimón,1* Adoración Valiente,1 María Ercibengoa,1 José M. García-Arenzana,1 and Emilio Pérez-Trallero1,2

Servicio de Microbiología, Hospital Donostia Paseo Dr. Beguiristain s/n, 20014 San Sebastián, Spain,1 and Departamento de Medicina Preventiva y Salud Pública, Universidad del País Vasco, Paseo Dr. Beguiristain s/n, 20014 San Sebastián, Spain2

Received 26 July 2005/Returned for modification 8 September 2005/Accepted 4 October 2005

The macrolide resistance determinants and genetic elements carrying the \textit{mef(A)} and \textit{mef(E)} subclasses of the \textit{mef} gene were studied with \textit{Streptococcus agalactiae} isolated in 2003 and 2004 from 7,084 vaginorectal cultures performed to detect carrier pregnant women. The prevalence of carriage was 18% (1,276 isolates), and that of erythromycin resistance 11.0% (129 of the 1,171 isolates studied). \textit{erm(B)}, \textit{erm(A)} subclass \textit{erm(TR)}, and the \textit{mef} gene, either subclass \textit{mef(A)} or \textit{mef(E)}, were found in 72 (55.8%), 41 (31.8%), and 12 (9.3%) erythromycin-resistant isolates, while 4 isolates had more than 1 erythromycin resistance gene. Of the 13 M-phenotype \textit{mef}-containing erythromycin-resistant \textit{S. agalactiae} isolates, 11 had the \textit{mef(E)} subclass gene alone, one had both the \textit{mef(E)} and the \textit{erm(TR)} subclass genes, and one had the \textit{mef(A)} subclass gene. \textit{mef(E)} subclass genes were associated with the carrying element \textit{mega} in 10 of the 12 \textit{mef(E)}-containing strains, while the single \textit{mef(A)} subclass gene found was associated with the genetic element \textit{Tn1207.3}. The nonconjugative nature of the \textit{mega} element and the clonal diversity of \textit{mef(E)}-containing strains determined by pulsed-field gel electrophoresis suggest that transformation is the main mechanism through which this resistance gene is acquired.

\textit{Streptococcus agalactiae} (Lancefield group B \textit{Streptococcus}) is a commensal bacterium of the human digestive and genital tracts and remains an important cause of perinatal morbidity and mortality. Prevention strategies based on the detection of vaginal and rectal colonization with \textit{S. agalactiae} followed by intrapartum administration of antibiotics to pregnant women have been demonstrated to reduce early-onset neonatal infection in the newborn (29, 32). \textit{S. agalactiae} also causes bacteremia and skin and soft tissue infections in adults, as well as other less-common but severe infections, such as endocarditis and meningitis (15, 30).

Due to the uniform susceptibility of \textit{S. agalactiae}, penicillin and ampicillin are currently the drugs of choice for the treatment and intrapartum prevention of neonatal infections, while clindamycin and erythromycin are recommended alternatives in cases of penicillin allergy (29, 32). There is concern about the increase of macrolide and clindamycin resistance in \textit{S. agalactiae} associated in some countries with increases in macrolide usage (10). In most recent studies performed in Spain and other countries, erythromycin resistance rates ranged between 10% and 20% (6, 10, 15).

Among the various mechanisms of macrolide resistance found in streptococci (20), the most frequently found in human isolates are methylation of streptococcal 23S rRNA at the erythromycin-binding nucleotide target due to \textit{erm} genes (erythromycin ribosome methylase) and a proton-dependent efflux of the drug mediated by the \textit{mef} genes (macrolide efflux) (13, 17). The presence of the \textit{erm} genes results in the macrolide-lincosamide-streptogramin B resistance phenotype, which may be constitutively or inducibly expressed. In \textit{S. agalactiae}, \textit{erm(B)} and \textit{erm(A)} subclass \textit{erm(TR)} genes are nearly always responsible for the macrolide-lincosamide-streptogramin B phenotype of macrolide resistance (6, 10, 15). Erythromycin resistance in streptococci mediated by the \textit{mef} genes confers resistance only to 14- and 15-membered-ring macrolides, resulting in the M phenotype of resistance (20, 33). The \textit{mef(A)} subclass gene was initially identified in \textit{Streptococcus pyogenes} and the \textit{mef(E)} subclass gene in \textit{Streptococcus pneumoniae}, and these two subclass genes are 90% identical. Both the \textit{mef(A)} and \textit{mef(E)} gene subclasses have been described for erythromycin-resistant \textit{S. agalactiae} clinical isolates (3).

In other streptococcal species, the \textit{mef(A)} subclass gene is carried in the defective transposon \textit{Tn1207.1} and \textit{mef(E)} in mega (macrolide efflux genetic assembly) (18, 26, 28). In \textit{S. pneumoniae}, mega can be inserted at different sites in the chromosome or into a transposon \textit{Tn1616}-like genetic element, forming a new composite named \textit{Tn2009}, which also contains the tetracycline resistance \textit{tet(M)} gene (12, 26). \textit{Tn2007.1} has been found in \textit{S. pneumoniae} inserted within the competence \textit{celB} gene (11, 28), and in \textit{S. pyogenes}, \textit{Tn1207.1} has been described as integrated into the conjugative transposon \textit{Tn1207.3} and into a conjugal \textit{tet(O)-mef(A)} element (4, 7, 9, 26).

The primary aim of the present study was to determine the macrolide resistance genes responsible for erythromycin resistance in \textit{S. agalactiae} isolated from pregnant women and to study the genetic elements carrying the \textit{mef} genes in the 13 M-phenotype erythromycin-resistant \textit{S. agalactiae} strains found. As a secondary aim, the presence of \textit{tet(M)} and other specific genes of the newly described transposon \textit{Tn2009} was also investigated to determine whether the ge-
etonic elements carrying the mef(E) gene were integrated into this transposon.

MATERIALS AND METHODS

Bacterial isolates. Throughout 2003 and 2004, all pregnant women in San Sebastián, Basque Country (Spain), were screened at weeks 35 to 37 for vaginal and anorectal carriage of S. agalactiae in Granada medium (27). Duplicate specimens were not included in the study. The characteristic red-orange colonies produced by S. agalactiae in Granada medium were tested for erythromycin susceptibility by the disk diffusion method according to CLSI (formerly NCCLS) methods and criteria (24). MICS of erythromycin and other antimicrobial agents were determined by the broth microdilution method using Sensititre microtiter trays (Sensititre; Trek Diagnostics Systems, West Sussex, England) and cation-adjusted Mueller-Hinton broth supplemented with 3% lysed horse blood and were interpreted according to the CLSI criteria (23).

DNA extraction. Specimens were stored at −80°C until DNA extraction. DNA extraction was performed using QIAamp spin columns (QIAGEN, Valencia, Calif.) according to the manufacturer’s instructions (ESSUM group B Streptococcus serotyping test; Bacterium AB, Umeå, Sweden).

PCR. The presence of the erythromycin resistance genes erm(B), erm(A) subclass erm(TR), and mef was studied with erythromycin-resistant isolates by PCR (Table 1). S. pneumoniae ATCC 700676 and ATCC 700677 were used as PCR-positive controls for the mef and erm(B) genes, respectively. S. agalactiae clinical isolate B222703 was used as a positive control in the PCR used to detect the mef(A) homologue, a gene encoding a protein that mediates resistance to macrolides and streptogramin B in Staphylococcus (18). These homologues are named mataA and mef according to the names provided in GenBank and in other streptococcal species are located downstream of mef(A) and mef(E), respectively (Fig. 1). After DNA extraction using QIAamp spin columns (QIAGEN, Chatsworth, CA), amplification was performed using the specific primers described in Table 1. PCRs were performed at a final volume of 50 μl using approximately 30 to 50 ng of genomic DNA as a template, 1 U of AmpliTaq Gold DNA polymerase (Roche, Branchburg, NJ), 200 μM deoxynucleoside triphosphates, 1/10 PCR buffer, 3 mM MgCl2, and 100 ng of each primer in a GeneAmp PCR system 2700 thermocycler (PE Applied Biosystems, Foster City, Califonia). PCR conditions for amplification of the these four genes comprised an initial denaturation step at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s, and elongation at 72°C for 1 min. A final elongation step at 72°C for 10 min was carried out after amplification cycles.

On mef-containing isolates, distinct regions of the genetic elements carrying the mef(A) and mef(E) genes were studied by means of PCR. The presence of transposon Tn1207.3 was studied using primers that hybridized with its orf5, orf9, and orf56 genes. orf8 is also present in Tn1207.1, but orf5 and orf56 are exclusive to transposon Tn1207.3 (Fig. 1).

The presence of mega was detected using specific primers that hybridized with its orf5 and orf7 genes on the basis of the S. pneumoniae sequences and mega and Tn2009. To study whether mega was inserted into a transposon Tn916-like element, a PCR with two specific sets of primers that hybridized with the mef(A) and mef(E) genes was studied by means of PCR. The presence of transposon Tn1207.3 was studied using primers that hybridized with its orf5, orf9, and orf56 genes. orf8 is also present in Tn1207.1, but orf5 and orf56 are exclusive to transposon Tn1207.3 (Fig. 1).

The presence of mega was detected using specific primers that hybridized with its orf5 and orf7 genes on the basis of the S. pneumoniae sequences and mega and Tn2009. To study whether mega was inserted into a transposon Tn916-like element, a PCR with two specific sets of primers that hybridized with the mef(A) and mef(E) genes was studied by means of PCR. The presence of transposon Tn1207.3 was studied using primers that hybridized with its orf5, orf9, and orf56 genes. orf8 is also present in Tn1207.1, but orf5 and orf56 are exclusive to transposon Tn1207.3 (Fig. 1).

The presence of mega was detected using specific primers that hybridized with its orf5 and orf7 genes on the basis of the S. pneumoniae sequences and mega and Tn2009. To study whether mega was inserted into a transposon Tn916-like element, a PCR with two specific sets of primers that hybridized with the mef(A) and mef(E) genes was studied by means of PCR. The presence of transposon Tn1207.3 was studied using primers that hybridized with its orf5, orf9, and orf56 genes. orf8 is also present in Tn1207.1, but orf5 and orf56 are exclusive to transposon Tn1207.3 (Fig. 1).

The presence of mega was detected using specific primers that hybridized with its orf5 and orf7 genes on the basis of the S. pneumoniae sequences and mega and Tn2009. To study whether mega was inserted into a transposon Tn916-like element, a PCR with two specific sets of primers that hybridized with the mef(A) and mef(E) genes was studied by means of PCR. The presence of transposon Tn1207.3 was studied using primers that hybridized with its orf5, orf9, and orf56 genes. orf8 is also present in Tn1207.1, but orf5 and orf56 are exclusive to transposon Tn1207.3 (Fig. 1).

The presence of mega was detected using specific primers that hybridized with its orf5 and orf7 genes on the basis of the S. pneumoniae sequences and mega and Tn2009. To study whether mega was inserted into a transposon Tn916-like element, a PCR with two specific sets of primers that hybridized with the mef(A) and mef(E) genes was studied by means of PCR. The presence of transposon Tn1207.3 was studied using primers that hybridized with its orf5, orf9, and orf56 genes. orf8 is also present in Tn1207.1, but orf5 and orf56 are exclusive to transposon Tn1207.3 (Fig. 1).

The presence of mega was detected using specific primers that hybridized with its orf5 and orf7 genes on the basis of the S. pneumoniae sequences and mega and Tn2009. To study whether mega was inserted into a transposon Tn916-like element, a PCR with two specific sets of primers that hybridized with the mef(A) and mef(E) genes was studied by means of PCR. The presence of transposon Tn1207.3 was studied using primers that hybridized with its orf5, orf9, and orf56 genes. orf8 is also present in Tn1207.1, but orf5 and orf56 are exclusive to transposon Tn1207.3 (Fig. 1).
**RESULTS**

**Erythromycin resistance and mechanism of resistance.** From January 2003 to December 2004, vaginorectal screening for *S. agalactiae* was performed on 7,084 pregnant women (3,629 in 2003 and 3,455 in 2004), and *S. agalactiae* was detected in 1,276 women, 657 in 2003 and 619 in 2004 (overall prevalence of carriage, 18.0%). As determined by disk diffusion, 129 of the 1,171 *S. agalactiae* isolates available for study (11.0%) were resistant to erythromycin.

Of the 129 erythromycin-resistant *S. agalactiae* isolates, 72 (55.8%) had the *erm* (B) gene, 41 (31.8%) had the *erm* (A) subclass *erm* (TR) gene, 12 (9.3%) had the *mef* gene, 3 (2.3%) had both the *erm* (B) and *erm* (A) subclass *erm* (TR) genes, and 1 (0.8%) isolate had both the *erm* (A) subclass *erm* (TR) and *mef* subclasses. Of the 12 isolates with only the *mef* gene, 11 had the *mef* (E) subclass gene and one had the *mef* (A) subclass gene. The broth microdilution method showed that all of these 13 *mef*-containing *S. agalactiae* isolates were susceptible to penicillin, trimethoprim-sulfamethoxazole, and rifampin and were resistant to tetracycline (Table 2).

**Detection of mega genetic element in *mef* (E)-containing strains.** In the 12 erythromycin-resistant *S. agalactiae* strains with the *mef* (E) gene, the *mel* and *orf5* genes from mega were also detected (Table 3). Two strains failed to amplify *orf7* from mega. The sequences of the *mef* (E), *mel*, *orf5*, and *orf7* genes of these *S. agalactiae* strains demonstrated a similarity of >99% to the corresponding sequences of mega and Tn2009 described for *S. pneumoniae*.

All *mef* (E)-containing isolates were also tetracycline resistant. Of these, 12 had the *tet* (M) gene and one had the *tet* (O) gene. In four of the *tet* (M)-containing strains, the *int*Tn and *orf24* genes of the transposon Tn916 family were detected, but no amplification was obtained with the primers designed to amplify the "orf5-orf6-tet(M)" fragment of transposon Tn2009 that was detected in the *S. pneumoniae* strain used as a control.

**Detection of Tn1207.3 in the *mef* (A)-containing strain.** In the single *S. agalactiae* isolate that contained the *mef* (A) subclass gene—strain B222296—the following genes of Tn1207.3 were detected: *mef* (A), *matA*, *orf8* (encoding an UmuC/MucB-like protein), *orf9* (encoding an unknown protein), and *orf56* (encoding a site-specific recombinase). All of these genes demonstrated a similarity of >99% to the sequences of the same corresponding fragments of Tn1207.3 described for *S. pyogenes* at GenBank. All these sequences were also found in a *mef* (A)-containing *S. pyogenes* clinical isolate used as control for these PCRs (data not shown).

**Serotyping and PFGE.** Serotyping showed that serotypes 1b and 11I were the most frequent among *mef* (E)-containing isolates (Table 2). By PFGE, the 11 strains that had the *mef* (E)
gene alone showed a different pattern (Fig. 2). Homology among their PFGE patterns was ≤80%.

**DISCUSSION**

A collection of 1,276 commensal *S. agalactiae* isolates isolated from healthy carrier pregnant women were studied for the prevalence of erythromycin resistance, the presence of the most frequent determinants of macrolide resistance, and the *mef*(A) and *mef*(E) genes carrying genetic elements. Both the prevalence of vaginorectal healthy carriers (18%) and the rate of macrolide-resistant isolates (11%) were in agreement with the results reported in other studies (10,15,19). The most frequent gene involved in erythromycin resistance was *erm*(B), followed by *erm*(A) subclass *erm*(TR), while, as found in other studies, the prevalence of *mef*-containing *S. agalactiae* was low (12,16,17). The high prevalence of *S. agalactiae* isolates containing the *erm*(B) and *erm*(A) subclass *erm*(TR) genes in carrier pregnant women is of concern, since for these patients preventative treatment with either macrolides or clindamycin could lead to therapeutic failures with a consequent risk of neonatal infection in the newborn.

Using serotyping and PFGE after restriction with Smal, no homology was found among *mef*(E)-containing strains, suggesting that, as occurs with other gram-positive bacterial species, horizontal transfer may be the main route through which this mechanism of erythromycin resistance is acquired (8,31). For several bacterial species found in the intestinal tract, including *Enterococcus* spp., viridans group streptococci, and other gram-positive and even gram-negative bacteria, the presence of the *mef* genes has been described (2,21). These species could act as reservoirs and donors of the *mef* genes of resistance to *S. agalactiae*.

Amplification by PCR of different open reading frame (ORF) genes of the *mef*(A)- and *mef*(E)-carrying genetic elements was performed to determine whether these elements, which have been found in other streptococci, were present in *S. agalactiae* strains. In the 12 strains with the *mef*(E) subclass gene, *mef*(E) was always associated with the *mel* resistance efflux gene described for *S. mutans*. In 10 strains, the *orf7* gene,

| Table 2. Serotypes and antibiotic MICs of 13 *S. agalactiae* strains with the M phenotype of macrolide resistance isolated from vaginorectally colonized pregnant women |
|---|---|---|---|
| Isolate | Strain no. | Isolation date (mo/day/yr) | Serotype | mef gene | Pen | Amp | Ery | Azi | Clr | Tet | Rif | Chl | Sxt | Cip | Lev |
| 1 | B223605 | 2/07/2003 | 1a | mef(E) | <0.03 | <0.25 | 4 | <0.25 | >4 | <1 | <4 | <0.5/9.5 | 1 | 1 |
| 2 | B220857 | 6/05/2003 | 1b | mef(E) | <0.03 | <0.25 | 4 | <0.25 | >4 | <1 | <4 | <0.5/9.5 | 1 | 1 |
| 3 | B221331 | 7/17/2003 | 1b | mef(E) | <0.03 | <0.25 | 4 | <0.25 | >4 | <1 | <4 | <0.5/9.5 | 1 | 1 |
| 4 | B222561 | 11/20/2003 | 1b | mef(E) | <0.03 | <0.25 | 4 | <0.25 | >4 | <1 | <4 | <0.5/9.5 | 1 | 1 |
| 5 | B222696 | 12/04/2003 | 1a | mef(A) | <0.03 | <0.25 | 4 | <0.25 | >4 | <1 | <4 | <0.5/9.5 | 1 | 1 |
| 6 | B220756 | 6/24/2004 | III | mef(E) | <0.03 | <0.25 | 4 | <0.25 | >4 | <1 | <4 | <0.5/9.5 | 1 | 1 |
| 7 | B220783 | 6/28/2004 | III | mef(E) | <0.03 | <0.25 | 4 | <0.25 | >4 | <1 | <4 | <0.5/9.5 | 1 | 1 |
| 8 | B220815 | 6/30/2004 | III | mef(E) | <0.03 | <0.25 | 4 | <0.25 | >4 | <1 | <4 | <0.5/9.5 | 1 | 1 |
| 9 | B220840 | 7/04/2004 | IV | mef(E) | <0.03 | <0.25 | 4 | <0.25 | >4 | <1 | <4 | <0.5/9.5 | 1 | 1 |
| 10 | B220867 | 7/06/2004 | III | mef(E) | <0.03 | <0.25 | 4 | <0.25 | >4 | <1 | <4 | <0.5/9.5 | 1 | 1 |
| 11 | B221495 | 9/03/2004 | II | mef(E) | 0.06 | <0.25 | 4 | <0.25 | >4 | <1 | <4 | <0.5/9.5 | 1 | 1 |
| 12 | B222129 | 11/04/2004 | V | mef(E) | <0.03 | <0.25 | 4 | <0.25 | >4 | <1 | <4 | <0.5/9.5 | 1 | 1 |
| 13 | B222261 | 11/18/2004 | Ib | mef(E) | <0.03 | <0.25 | 4 | <0.25 | >4 | <1 | <4 | <0.5/9.5 | 0.5 | <0.5 |

*Pen, penicillin; Amp, ampicillin; Ery, erythromycin; Azi, azithromycin; Clr, clindamycin; Tet, tetracycline; Rif, rifampin; Chl, chloramphenicol; Sxt, trimethoprim-sulfamethoxazole; Cip, ciprofloxacin; Lev, levofloxacin.*

**Table 3. PCR amplification of specific DNA fragments and tet(M) and tet(O) genes in M-phenotype erythromycin-resistant *S. agalactiae* strains**

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Strain no.</th>
<th>ORFs of Tn1207.3</th>
<th>ORFs of megal</th>
<th>ORFs of Tn916 and Tn2009</th>
<th>Presence of:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>B223605</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>2</td>
<td>B220857</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>3</td>
<td>B221331</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>4</td>
<td>B222561</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>B222696</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>B220756</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>7</td>
<td>B220783</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>8</td>
<td>B220815</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>9</td>
<td>B220840</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>10</td>
<td>B220867</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>11</td>
<td>B222129</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>12</td>
<td>B222261</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>
located upstream of mef(E) at the 5’ end of the mega sequence, and the orf5 gene, located at the 3’ end of the mega sequence, were also detected. Adding the four DNA fragments of mega sequenced in mef(E)-containing S. agalactiae strains, orf7, mef(E), mel, and orf5, nearly half of the mega element was sequenced, showing a similarity of >99% with the mega sequence of S. pneumoniae at GenBank. The detection of different parts of the mega element, together with the mef(E) and mef genes, strongly suggested that, as occurs in S. pneumoniae and other streptococcal species containing the mef(E) subcell gene (1, 12, 18, 26), in the majority of the mef(E)-containing S. agalactiae isolates studied the mef(E) subcell gene was carried in mega.

The absence of the intTn and orf24 genes in 8 of the 12 mef(E)-containing strains ruled out the presence of a Tn916-like element. In the four remaining mef(E)-containing S. agalactiae strains in which the intTn and orf24 genes were detected, the failure to demonstrate the location of mega upstream of tet(M) indicated that mega was not carried, at least not in the same orientation as that described for the Tn2009-like transposon.

Erythromycin-resistant S. agalactiae isolates with the M phenotype usually have the mef(E) subcell gene, while mef(A) subcell-containing isolates are rarely found (3). We found only one strain with mef(A), in which the matA and the orf8 genes described for the Tn1207.1 genetic carrying element were also detected. The detection of the orf9 and orf56 genes of the 5’- and 3’-end regions of transposon Tn1207.3 in this mef(A)-containing strain indicated that Tn1207.1 was integrated into the conjugative transposon Tn1207.3. In addition, the detection of the tet(M) gene and not of tet(O) in this erythromycin- and tetracycline-resistant strain ruled out the carriage of Tn1207.1 into the recently described tet(O)-mef(A) element (7).

As far as we know, this is the first report of the presence of mega and Tn1207.1 integrated into Tn1207.3 in S. agalactiae. These two genetic elements have been described previously for other pathogenic and non-pathogenic streptococcal species, such as S. pneumoniae, S. pyogenes, and viridans group streptococci (4, 12, 18, 26, 28).

In conclusion, in S. agalactiae isolated from pregnant carrier women, the rates of prevalence, erythromycin resistance, and determinants of macrolide resistance found were similar to those described in other studies. In erythromycin-resistant S. agalactiae with the M phenotype, the presence of the mef(E) and mef(A) genes was associated with the mega- and Tn1207.3-carrying elements, respectively. The clonal diversity of mef(E)-containing strains and the described lack of conjugation of the mega element (18, 26) suggest that transformation might be the main mechanism through which this genetic element of resistance is acquired.

REFERENCES


