Vancomycin-resistant enterococci (VRE) have been reported to be present in humans, chickens, and pigs in Malaysia. In the present study, representative samples of VRE isolated from these populations were examined for similarities and differences by using the multilocus sequence typing (MLST) method. Housekeeping genes of Enterococcus faecium \((n = 14)\) and Enterococcus faecalis \((n = 11)\) isolates were sequenced and analyzed using the MLST databases eBURST and goeBURST. We found five sequence types (STs) of \(E.\ faecium\) and six STs of \(E.\ faecalis\) existing in Malaysia. Enterococcus faecium isolates belonging to ST203, ST17, ST55, ST79, and ST29 were identified, and \(E.\ faecium\) ST203 was the most common among humans. The MLST profiles of \(E.\ faecium\) from humans in this study were similar to the globally reported nosocomial-related strain lineage belonging to clonal complex 17 (CC17). Isolates from chickens and pigs have few similarities to those from humans, except for one isolate from a chicken, which was identified as ST203. \(E.\ faecalis\) isolates were more diverse and were identified as ST4, ST6, ST87, ST108, ST274, and ST244, which were grouped as specific to the three hosts. \(E.\ faecalis\), belonging to the high-risk CC2 and CC87, were detected among isolates from humans. In conclusion, even though one isolate from a chicken was found clonal to that of humans, the MLST analysis of \(E.\ faecium\) and \(E.\ faecalis\) supports the findings of others who suggest VRE to be predominantly host specific and that clinically important strains are found mainly among humans. The infrequent detection of a human VRE clone in a chicken may in fact suggest a reverse transmission of VRE from humans to animals.

**MATERIALS AND METHODS**

**Properties and origin of bacteria.** We selected VRE isolates from among those obtained in the course of a few epidemiological studies on VRE that were carried out in several states within Peninsular Malaysia over a period of 4 years (2005 to 2009). Due to limited resources, we chose 25 representative vancomycin-resistant \(E.\ faecalis\) \((n = 11)\) and \(E.\ faecium\) \((n = 14)\) isolates among 140 isolates originating from colonized chickens, pigs, and humans. Clinical isolates from human infections \((E.\ faecium, n = 2; \text{and} \ E.\ faecalis, n = 1)\) were generously donated by the Institute of Medical Research (IMR), Kuala Lumpur, Malaysia. These isolates were among the

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very few isolates available from clinical infection. All isolates used in this study were vanA gene positive and have similar levels of vancomycin resistance (MIC $\geq$ 32 μg/ml). The study design, the evidence for ethical conduct in our human sampling, the bacterial analysis, and determination of species, including resistance van gene determination and vancomycin resistance level, have been published elsewhere (21, 22, 26).

**RESULTS**

**Amplification and sequencing of housekeeping genes.** We performed the amplification of the internal gene fragments of seven *E. faecium* and *E. faecalis* housekeeping genes based on established multilocus sequence typing (MLST) schemes (www.mlst.net). The primers for the housekeeping genes (gdh, gdh, pstS, gki, aroE, xpt, and ygiL) and *E. faecalis* (gki, gki, pstS, purK, ddl, atpAn, and adk) were described by Homan et al. (16) and Ruiz-Garbajosa et al. (29), respectively. Genomic DNA was extracted from fresh colonies grown on BHI agar (Difco). The DNeasy blood and tissue DNA extraction kit (Qiagen, Germany) was used to extract genomic DNA. The HotStar Taq plus master mix kit (Qiagen, Germany) was used to amplify the housekeeping genes, using standard PCR. The genes were then purified with the MEGAquick-spin PCR purification system (Intron Biotechnology, South Korea), and direct sequencing was done at Macrogen Inc., South Korea.

**Sequence analysis.** The unique sequences of the seven loci were analyzed using the *E. faecium* and *E. faecalis* databases (www.mlst.net) to determine their allelic profile (or genotype) and were assigned a sequence type (ST). Isolates with identical allelic profiles are considered members of a single clone or lineage, isolates that differed in no more than one locus are referred to as single-locus variants (SLV), and isolates that vary in more than two and three loci are classified as double-locus variants (DLV) and triple-locus variants (TLV), respectively. Isolates published in the MLST database for both *E. faecium* and *E. faecalis* were used for comparison. Using eBURST version 3 and goeBURST version 1.2.1 software (30) (http://web.ist.utl.pt/aplf/arXiv/10-FVMRC-PHY.pdf), groups of isolates that differed in no more than one of the seven loci (SLV) of a founder isolate were clustered into the same clonal complex (CC). Neighbor-joining trees were generated using the MLST website Batch Query option. This option allows for the construction of an unweighted-pair group method using average linkages (UPGMA) dendrogram based on the pairwise differences in the allelic profiles of isolates from the study.

**RESULTS**

**MLST of *E. faecium* isolates.** The clonal lineages based on MLST of 14 isolates identified six different STs: ST203, ST55, ST29, ST79, ST57, and ST17. Five (71%) human isolates (n = 7) (including a clinical isolate) were ST203, one clinical isolate belonged to ST17, and one human isolate belonged to ST79. All strains (100%) from the pigs (n = 3) were ST55, and isolates from the chickens (n = 4) were ST203, ST55, ST57, and ST29. Overall, six (43%) isolates were ST203, four (28%) were ST55, and the remaining four *E. faecium* isolates were distinct (Table 1). Twelve of 14 (86%) isolates clustered according to host species (Fig. 1).

Using the goeBURST algorithm (31), we found one locus (i.e., *ddl*) difference between isolates of ST29 and ST57 and two locus variations (i.e., *atpA* and *pstS*) between ST17, ST203, and ST55, and there were more than three loci variations among isolates of ST57, ST17, and ST79 (Table 1).

We determined the evolutionary relationships by analyzing in eBURST the MLST profile of all STs available in the database. In eBURST, the registered isolates belong to five major clonal com-

![FIG 1](http://aem.asm.org) A neighbor-joining tree showing evolutionary relationships of *E. faecium* isolates from clinical cases and colonized humans, chickens, and pigs.
TABLE 2 MLST profiles of \textit{E. faecalis} isolates from humans, pigs, and chickens\textsuperscript{a}

<table>
<thead>
<tr>
<th>Isolate</th>
<th>CC</th>
<th>ST</th>
<th>Source</th>
<th>Allele numbers of indicated housekeeping gene</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>\textit{gdh} \textit{gyd} \textit{pstS} \textit{gki} \textit{aroE} \textit{xpt} \textit{yqiL}</td>
</tr>
<tr>
<td>S515</td>
<td>87</td>
<td>87</td>
<td>Human</td>
<td>28 4 8 3 8 1 3</td>
</tr>
<tr>
<td>W54</td>
<td>2</td>
<td>6</td>
<td>Human</td>
<td>12 7 3 7 6 1 5</td>
</tr>
<tr>
<td>Clinical99</td>
<td>SS</td>
<td>4</td>
<td>Human</td>
<td>8 7 7 5 4 4 1</td>
</tr>
<tr>
<td>Chicken129</td>
<td>SS</td>
<td>244</td>
<td>Chicken</td>
<td>11 5 4 16 11 13 49</td>
</tr>
<tr>
<td>Chicken114</td>
<td>SS</td>
<td>244</td>
<td>Chicken</td>
<td>11 5 4 16 11 13 49</td>
</tr>
<tr>
<td>Chicken7</td>
<td>SS</td>
<td>244</td>
<td>Chicken</td>
<td>11 5 4 16 11 13 49</td>
</tr>
<tr>
<td>Chicken13</td>
<td>SS</td>
<td>244</td>
<td>Chicken</td>
<td>11 5 4 16 11 13 49</td>
</tr>
<tr>
<td>Chicken73</td>
<td>SS</td>
<td>244</td>
<td>Chicken</td>
<td>11 5 4 16 11 13 49</td>
</tr>
<tr>
<td>Pig18</td>
<td>SS</td>
<td>274</td>
<td>Pig</td>
<td>31 7 16 1 27 10 23</td>
</tr>
<tr>
<td>Pig43</td>
<td>SS</td>
<td>274</td>
<td>Pig</td>
<td>31 7 16 1 27 10 23</td>
</tr>
<tr>
<td>Pig45</td>
<td>SS</td>
<td>108</td>
<td>Pig</td>
<td>1 2 30 13 37 22 32</td>
</tr>
</tbody>
</table>

\textsuperscript{a} CC, clonal complex; SS, singleton; ST, sequence type; \textit{gdh}, glucose-6-phosphate dehydrogenase gene; \textit{gyd}, glyceraldehyde-3-phosphate dehydrogenase gene; \textit{pstS}, phosphate ATP-binding cassette transporter gene; \textit{gki}, glucokinase gene; \textit{aroE}, shikimate-5-dehydrogenase gene; \textit{xpt}, xanthine phosphoribosyltransferase gene; \textit{yqiL}, acetyl coenzyme A acetyltransferase gene.

plexes (CC) in the order of their size: CC17, CC9, CC22, CC5, and CC94. Accordingly, ST57, ST29, and ST79 identified by this study are part of CC9, ST55 is part of CC22, and ST17 and ST203 are part of the globally dispersed and major outbreak clonal lineage CC17.

The housekeeping gene \textit{purK} allele 1 (\textit{purK1}) often affiliated with epidemic \textit{E. faecium} was detected in seven (50%) isolates. Except for one chicken isolate, all \textit{purK1} isolates were from humans (Table 1).

MLST of \textit{E. faecalis}. Eleven \textit{E. faecalis} isolates were classified into six STs: ST244, ST274, ST108, ST87, ST6, and ST4. The most abundant ST was ST244 (45%) followed by ST274 (18%), and the remaining STs were detected once. All chicken isolates (n = 5) were ST244, two of the pig isolates (n = 3) were ST274, and one pig isolate was ST108. Human isolates (n = 3) were completely diverse, with different STs. The details of the MLST profile of each isolate are provided in Table 2.

The six STs showed a high level of genetic variation and thus failed to be clustered by eBURST. Sequence types with three or fewer locus variations form a group, but STs identified in this study had more than three loci variations, and thus none of them formed a group.

Genetic similarities and differences of human and animal isolates are presented in Fig. 2. Seven (64%) isolates were clustered according to their respective animal hosts. The genetic differences were quantified by comparing the concatenated sequences of the seven housekeeping genes for respective STs. The differences are shown as branch length in Fig. 2.

**DISCUSSION**

We detected six STs of \textit{E. faecium} (ST203, ST55, ST29, ST79, ST57, and ST17) in this study. The most frequent STs observed were ST203 and ST55, which belong to the widespread nosocomial lineage CC17 (6, 32–34). ST203 was a unique allelic type of \textit{E. faecium} that caused outbreaks in hospitals in Korea (35, 36), Germany (37, 38), and China (39, 40). At the time this paper was written, there were 43 entries of this ST in the MLST database, of which more than 60% were reported from Asia-Pacific countries. Our isolates did not match any isolates from hospital outbreaks, sporadic infections, and human fecal colonization from Singapore, our closest neighbor, where \textit{E. faecium} isolates of ST280, ST117, ST18, and ST64 have been reported (41). ST280, ST64, and ST117 were closest (with SLV) to the clinical isolate from human from this study (ST17).

The database shows that the presence of ST203 has also been reported in healthy persons, on pig farms, in slaughterhouses, and in the environment (sewage, rivers). In the present study, most clinical and healthy human isolates and one chicken isolate were of ST203. The isolation of ST203 in chicken has never been reported; nevertheless, this finding is not alarming, because Willems and van Schaik (42) showed that 9% of \textit{E. faecium} isolates from infected humans (including those of nosocomial origin) and 8% of isolates from the community (healthy humans) share similar STs with chicken isolates. Other VRE strains that have been over-whelmingly reported as causing nosocomial outbreaks have also, in some instances, been detected on farm animals and in companion animals (13, 43). For example, within the CC17, ST17 (also reported in this study from one clinical isolate) and ST18, which

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**FIG 2** A neighbor-joining tree showing the evolutionary relationships of \textit{E. faecalis} isolates from clinical cases and colonized humans, chickens, and pigs.
are two of the most widespread and predominant strains causing nosocomial infection, have been isolated from pigs or pig environments (44) and from chicken-based products (43, 45). In addition, ST132, another strain within CC17 causing clinical infections in humans, was isolated from a pig in the Netherlands (46).

We believe that it is possible that the chicken acquired the ST203 E. faecium strain from contaminated environments, other animals, or humans working on or visiting the farm. Our assertion is supported by an experimental study that demonstrated transmissibility of VRE of human origin to broiler chickens (47). Furthermore, human-adapted VRE strains have been recovered from various food and urban water sources (43, 48–50). Another report showed a high prevalence of multidrug-resistant E. faecalis and E. faecium in houseflies (51). It is also possible that wild animals, especially rodents (52), birds, and flies, acquire these environmental contaminants and pass them on mechanically or via their excreta to grazing land or to livestock feed materials (53). Finally, a recent study on Glaucous gulls in poorly inhabited remote areas of Point Barrow, AK, that found vancomycin-resistant E. faecium belonging to CC17 lineage lends further support to the assertion that the dispersal of resistant strains can occur via contamination of the environment or from anthropogenic sources (54).

Another human strain isolated in our study is ST79, which has TLVs with ST17 and belongs to CC9. The clone is infrequently reported as a clinical strain and has been described as a chicken strain by Freitas et al. (55). However, this ST was not found in the chickens in the present study.

Most (86%) of the isolates in our study clustered according to their host: six of 7 human isolates belonged to CC17, all pig isolates were clonal and clustered in CC22, and two of the four chicken isolates belonged to CC9. This is consistent with another study that reported 70 to 90% of isolates from swine, chicken, and veal calves had STs that were predominantly host specific (42). Homan et al. (16) concluded that a distinctive genetic lineage exists between VRE strains causing nosocomial infections and lineages composed of the community and animal-derived isolates. The author further concluded that the relationship is the result of long-term coevolution of the bacterium and the host. However, other authors also suggested that some VRE strains are shared between different hosts, and these are usually the nonepidemic strains. In our study, E. faecium from the pigs and one chicken shared ST55. Indeed, Top et al. (56) indicated that ST55 can be isolated in various sources. The strains of ST29 and ST37 that were isolated from chickens in this study were also reported to be isolated from people in the community and from pigs in Brazil, France, Australia, and Belgium. ST29 and ST37 belong to CC9 that has been identified as a global chicken lineage complex (55).

The housekeeping gene purK was suggested as a marker for the epidemic vancomycin-resistant E. faecium genotypes (57). Genotypes characterized by the presence of esp, purK1, and ampicillin resistance are the most frequent among outbreak-associated isolates (6). In the present study, six (86%) isolates from humans (clinical cases and healthy humans) and one (25%) from a chicken were purK1 type. Our finding is supported by a similar study that reported a high prevalence (73%) of purK1 isolates in both clinical and community sources (43). Novais et al. (50) also detected VRE with purK1 in rivers and sewage, suggesting that the contamination of the environment may lead to the colonization of livestock with this strain. Others have reported ST443 isolated from swine that harbor the purK1 allele (55). ST79 isolated from a healthy human in this study had purK6. This allele is more similar to nontypeable purK alleles than to the clinical purK1 (58). CC9 strains associated with purK6 or purK3 are reported to belong to a widely distributed poultry lineage (55). Two isolates from chickens in this study were purK2 and purK3, while the isolates from pigs were all purK2. Other authors had shown that purK3 is prevalent (93%) in chicken (live and carcasses) (43). On the contrary, Coque et al. (58) reported that outpatients were the main source of purK3. The present study revealed that the outbreak-associated purK1 E. faecium isolates were more common in humans than in chickens or pigs. Our findings further emphasized the role of the community as the predominant reservoir of enterococci for human infections.

E. faecalis. A high genetic diversity of E. faecalis isolated from hospitalized patients, surveillance samples, animals, and food has been described in earlier studies (6, 29). A study on E. faecalis population structure detected highly diverse clones with predominance of CC2, CC9, CC10, CC21, CC40, CC87, and the singleton ST16 (29). The present analysis also confirmed the diverse nature of this species. The genetic diversity was noted in human and pig isolates but not in chicken isolates. The isolates from chickens were clonal (ST244), suggesting that E. faecalis isolates were derived from a single clone that was specific to chickens. This observation was inconsistent with those authors who described a lack of apparent host specificity in E. faecalis population (46).

Strains of CC2, CC9, and CC87 are linked to nosocomial infection and community colonization (6, 46, 59). Interestingly, two of the high-risk global clonal complexes of E. faecalis (CC2 and CC87) were detected in this study from apparently healthy humans, and the clinical isolate in our study was not part of a known clonal complex in the MLST database. Strains of ST6 (CC2) were reported from France, Netherlands, Spain, Poland, Portugal, and the United States from hospitalized patients (46) and the community (60, 61). This suggests that the E. faecalis ST6 isolated from the healthy humans in this study is a common human strain. The clinical isolate obtained in the course of this study, E. faecalis ST4, was reported from hospitalized patients in Thailand and Japan (62). This may suggest that ST4 is a common nosocomial strain in Asian countries.

Conclusions. Our study was limited in the number of isolates that could be analyzed; therefore, inferences from this study should be made with this knowledge in mind. However, we believe that this research has provided an insight into the MLST profiles of vancomycin-resistant E. faecium and E. faecalis from humans, chickens, and pigs in Malaysia. At least six sequence types for E. faecium (ST203, ST17, ST55, ST29, and ST79) and E. faecalis (ST4, ST6, ST87, ST108, ST274, and ST244) exist. Clustering analysis showed that more than 80% of the isolates were host specific. Isolates from healthy individuals had a close similarity to clinical isolates, and there was high dissimilarity between animal and human isolates in general. Comparison of MLST profiles from this study in the global database showed that six human E. faecium isolates were derivatives of the epidemic CC17 and two E. faecalis human isolates were derived from epidemic CC2 and CC87. Our findings revealed little evidence to support the view that VRE from pigs and chickens were colonizing humans in Malaysia.

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