Correlation between Infective Factors and Antibiotic Resistance in Enterococci Clinical Isolates in West of Iran

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The present study was done to scrutinize the possible relation between infective genes and antimicrobial resistance in Enterococcus faecalis and Enterococcus faecium. Considering the fact that the presence of recognized infective determinants among clinical isolates may promote the emergence of infections and persistence of Enterococci in hospital settings, which can lead to an increase in antimicrobial resistance. 175 E. faecalis and 67 E. faecium isolated from clinical specimens were used. The isolates were identified, and then antibiotic susceptibility testing was performed. The MIC of vancomycin and teicoplanin were determined by broth microdilution method. The presence of infective genes esp, hyl and asa1 was scrutinized using PCR. Of the 280 enterococcal isolates, 175 (62.5%) isolates were identified as E. faecalis, 67 (24%) as E. faecium and 38 (13.5%) as Enterococcus spp. The results of the antibiotic susceptibility testing showed resistance rates of 5% and 73% to vancomycin and teicoplanin in E. faecalis and E. faecium isolates, respectively. The statistical analysis showed that the esp infective gene has significant associations with ciprofloxacin, erythromycin and tetracycline in E. faecium and with chloramphenicol in E. faecalis strains; the hyl with teicoplanin and vancomycin in E. faecium strains; and also asa1 with vancomycin in E. faecium and with ampicillin and chloramphenicol in E. faecalis strains. Regarding the relationships between virulence genes and antibiotic resistance in strains of E. faecalis and E. faecium, detection of infective factors associated with invasive diseases has become a major issue of concern.

Key Words: Enterococcus; Anti-Bacterial Agents; Virulence

INTRODUCTION

Enterococci bacteria are facultative anaerobic gram-positive cocci, which are considered part of the normal flora in humans and animals. However, these microorganisms may be the cause of several serious systematic infections too.1-3 The two most common Enterococcus species, E. faecalis and E. faecium are responsible for 80-90% and 5-10% of human enterococcal infections, respectively.5 E. faecalis is the most common isolate of nosocomial infections, but newly, due to increasing resistance to some antimicrobial agents, especially vancomycin, E. faecium isolates are also being considered.1 It has been shown that separate lineages of E. faecalis and E. faecium are leading causes of the large number of the multidrug-resistant enterococcal infections. According to several investigations, CC17 and closely related strains are the main agents of most hospital-acquired infections association with E. faecium.5 Although these organisms lack strong virulence factors, they may have an innate resistance/tolerance to many important antibacterial agents such as, cephalosporins, cotrimoxazole, and low levels of penicillin and aminoglycosides, polymyxin, lincomamide, trimethoprim-sulfamethoxazole, monobactams, streptogramin. They are also able to acquire resistance to penicillin, chloramphenicol, tetracyclines, aminoglycosides and vancomycin.3,6-8

Pathogenicity and increased risk of acquisition of enter-
Enterococcal infections are linked to antimicrobial resistance and expression of virulence factors including, adhesion factors, translocation, and immune evasion. In hospital settings, infective potential of enterococci can be due to selective the advantages conferred by their antibiotic resistance. Furthermore, there is great concern about infectious diseases because of spreading antimicrobial resistance genes. The most important infective agents of enterococci which have been identified, include: aggregation substances (asa), cytolysin (cyl), hyaluronidase (hyl), the enterococcal surface protein (esp) and gelatinase (gelE).1-5 It has been found that some of virulence factors such as, agg, esp and cyl genes, located on 153-kb pathogenicity island.1-5 Persistence of enterococci in the hospital setting may be associated with their virulence factors.1-5 Based on several studies, the virulence factors of gelatinase, aggregation substance and cytolysin have not been found in E. faecalis in contrast to E. faecalis. On the other hand, esp and hyl have been found in both E. faecalis and E. faecium.12

Furthermore, clinical isolates of enterococci have virulence determinants that may result in promoting the emergence of infections and the persistence of these organisms in hospital settings which consequently can lead to increased resistance.15 This study was designed to scrutinize some virulence genes including asa1 (aggregation substance), esp (enterococcal surface protein), gelE (gelatinase), hyl (hyaluronidase) in clinical isolates of E. faecalis and E. faecium to investigate possible correlations between virulence and antibiotic resistance.

MATERIALS AND METHODS

1. Identification of enterococcal isolates

One hundred and seventy-five E. faecalis and sixty-seven E. faecium strains were collected from discrete clinical samples submitted to three teaching hospitals (including Beheshti, Besat and Farschian Hospitals) located in Hamedan, Iran, from December 2012 to May 2014. The origins of the isolates were as follows: urine 200 (82.6%), endotracheal aspirate 23 (9.5%), blood 8 (3.3%), Skin soft tissue 6 (2.5%), and body fluids 5 (2.1%). The isolates were pinpointed using routine microbiological methods. Then, PCR targeting D-alanine- D-alanine ligases for E. faecalis (ddl E. faecalis) and E. faecium (ddl E. faecium) was used to confirm phenotypic speciation.15

2. Detection of E. faecalis and E. faecium species by PCR assay

Firstly, enterococcal DNA was extracted by boiling.16 Then, a mastermix PCR Kit ([PCR 2X Taq premix Mastermix, Arianous Biotec Co.] was used to perform the PCR reaction. PCRs were performed with specific primers for each gene (Table 1) with some modifications to Kariyama’s protocol12 using Eppendorf and Bioread thermocycler in a final volume of 20 µL. The thermal cycle program was performed by initial denaturation at 95°C for 5 min, followed by amplification in 30 cycles of denaturation at 95°C for 30 s, annealing at 52.5°C for 30 s and elongation at 72°C for 1 min, and a final extension at 72°C for 10 min. E. faecalis ATCC 29212 and E. faecium BM4147 were used as quality control strains.

3. Antibiotic susceptibility testing

The antimicrobial susceptibilities of 175 E. faecalis and 67 E. faecium strains were examined by using the disk agar diffusion (DAD) method in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines.17,18 Erythromycin (15 µg), Tetracycline (30 µg), Ciprofloxacin (5 µg), Vancomycin (30 µg), Teicoplanin (30 µg), Norfloxacin (10 µg), Nitrofurantoin (300 µg), Quinopristin-Dalfopristin (Synercid (15 µg)) (Mast Co., UK), Chloramphenicol (30 µg), Gentamicin (10 µg), Linezolid (30 µg), and Ampicillin (10 µg) (HiMedia Mumbai Co., India) were used for anti-microbial susceptibility testing (AST).

In addition, minimum inhibitory concentrations (MIC) of the glycopeptide antibiotics i.e. vancomycin and teicoplanin (Sigma-Aldrich, Poole, Co., UK) against the E. faecalis and E. faecium isolates were determined using the microdilution broth method.17,18 E. faecalis ATCC 29212 (Vancomycin sensitive), E. faecalis ATCC 51299 (vanB positive), E. faecalis E206 (vanA positive) were used as quality control.

| Gene targets | Primer sequences (5’ to 3’) | Amplicon/product size (bp) | References |
|--------------|-----------------------------|----------------------------|------------|
| asa1         | F: GCAGCATTACGGAACATGTA     | 375                        | 13         |
|              | R: TAAGAAAGAACAATCACCAGA    |                            |            |
| hyl          | F: ACAGAAGAGCTCAGGAAGATG    | 276                        | 19         |
|              | R: GACCTGAGCTCAGGTTG        |                            |            |
| esp          | F: AAGTTTCATCTTTGAGTTTGG    | 510                        | 13         |
|              | R: AATTTGATTTCATGATTTGG     |                            |            |
| ddl E. faecalis | F: ATCAAGTACTAGTTGTTTATTAG | 941                        | 13         |
|              | R: ACGATTCAAGGCTAATCAATTG   |                            |            |
| ddl E. faecium | F: TTAGGGACGACGACATGAGCG   | 658                        | 13         |
|              | R: TATGACAGCGACTCCGATTCC    |                            |            |
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4. Detection of infective genes esp, hyl, and asa; by PCR

Multiplex PCR and single PCR were used for the identification of esp, asa, and hyl virulence determinants using specific primers for each gene with some modifications on Vankercovn's protocol (Table 1).

Briefly, the final volume multiplex PCR reaction for genes esp and asa, was 25 μL and for hyl gene was 20 μL. The PCR reactions were done for both mixtures on an Eppendorf and Biorad thermocycler (ASTEC Co., Japan) with an initial denaturation at 95°C for 10 min, 30 cycles of amplification (denaturation at 94°C for 1 min, annealing at 56°C for 1 min, and extension at 72°C for 1 min), and a final extension at 72°C for 10 min. The E. faecalis ATCC 29212 (asa positive), E. faecium C68 (hyl and esp positive) were used as quality control.

5. Statistical analysis

Correlation between antibiotic susceptibility patterns and occurring virulence genes was analyzed statistically using the Chi-Square test; the differences were considered significant for \( p < 0.0012 \) using the Bonferroni correction based on several primary comparisons. In addition, significant differences for simultaneous occurrence of virulence genes in enterococci strains was determined using Fisher's Exact test with a significance level of \( p < 0.05 \). All tests were performed using SPSS software (version 19).

RESULTS

1. Isolation of enterococci strains

Using biochemical methods, of the 280 enterococcal isolates, 190 (67.8%), 75 (26.8%) and 15 (5.4%) isolates were recognized as E. faecalis, E. faecium and Enterococcus spp., respectively. Among the determined presumptive E. faecalis, E. faecium isolates, 175 (62.5%) E. faecalis and 67 (24%) E. faecium strains were confirmed using the PCR method (Fig. 1). Therefore, a total of 38 strains (13.5%) remained as a part of the Enterococcus genus and were excluded from the current study. Urine samples were the highest isolation source of E. faecalis and E. faecium strains. Among 175 E. faecalis strains, 153 isolates and among 67 E. faecium strains, 48 isolates were isolated from urine samples; followed by tracheal sample with 17 isolates (11 strains were determined as E. faecalis and 6 strains as E. faecium).

The highest prevalence of E. faecium strains was observed in wounds and other organs samples (abscess and pulmonary secretions); among 12 strains isolated from wounds and other organs, 8 strains belonged to E. faecium (Table 2).

2. Antimicrobial susceptibility testing

Table 3 shows the susceptibility patterns of 67 E. faecium and 175 E. faecalis strains to 12 commonly used antibiotics. Resistance to the majority of antibiotics except for chloramphenicol, tetracycline, and quinopristin-dalfopristin was higher in E. faecium isolates compared to E. faecalis isolates. However, they both showed good rates of sensitivity to linezolid (100%), nitrofurantoin and chloramphenicol (74.6%). In fact, no resistance to the linezolid antibiotic was observed among E. faecalis and E. faecium strains; and all isolates of E. faecalis were susceptible to nitrofurantoin. Among 175 E. faecalis strains, the greatest resistance was observed for Synercid antibiotic with frequency of 167 (95.4%) isolates; followed by tetracycline with 154 (88%), erythromycin with 109 (62.3%), ciprofloxacin with 69 (39.4%), gentamicin with 63 (36%), Norfloxacin with 58 (33%), chloramphenicol with 57 (32.6%), vancomycin and teicoplanin with 9 (5%) and ampicillin with 6 (3.4%) isolates. Among 67 E. faecium isolates, the greatest resistance was observed for gentamicin with a frequency of 62 (92.5%) strains, followed by erythromycin with 58 (86.6%), norfloxacin with 56 (83%), ciprofloxacin with 54 (80.6%), vancomycin with 51 (76%), tetracycline and teicoplanin with 49 (73%), Synercid with 47 (70%), ampicillin with 42 (62.7%), nitrofurantoin with 17 (25.4%),
TABLE 3. Antibiotic resistance behavior of *Enterococci* isolates using disk diffusion method

| Antimicrobial agent | *E. faecalis*=175 | *E. faecium*=67 | Total=242 |
|---------------------|-------------------|-----------------|-----------|
| Vancomycin          | 166 0 9           | 16 0 51         | 182 0 60  |
| Teicoplanin         | 166 0 9           | 18 0 49         | 184 0 58  |
| Ampicillin          | 169 - 6           | 25 - 42         | 194 - 48  |
| Tetracycline        | 17 4 154          | 14 4 49         | 31 8 203  |
| Ciprofloxacin       | 64 42 69          | 0 13 54         | 64 55 123 |
| Norfloxacin         | 110 7 58          | 0 11 56         | 110 18 114|
| Erythromycin        | 46 20 109         | 0 9 58          | 46 29 167 |
| Synercid            | 8 0 167           | 16 4 47         | 24 4 214  |
| Chloramphenicol     | 95 23 57          | 50 10 7         | 145 33 64 |
| Gentamicin          | 98 14 63          | 1 4 62          | 99 18 125 |
| Nitrofurantoin      | 175 0 0           | 50 0 17         | 225 0 17  |
| Linezolid           | 175 0 0           | 67 0 0          | 242 0 0   |

TABLE 4. The prevalence of virulence determinants among *E. faecalis* and *E. faecium* clinical isolates

| Clinical samples | *E. faecalis* virulence genes (N=175) | *E. faecium* virulence genes (N=67) |
|------------------|--------------------------------------|-------------------------------------|
|                  | esp (N=126)  hyl (N=93)  asa₁ (N=152) | esp (N=41)  hyl (N=37)  asa₁ (N=48) |
| Urine            | 126 93 152                                  | 41 37 48                              |
| Treacheal        | 4 2 8                                       | 3 3 6                                |
| Blood            | 4 3 5                                       | 2 2 3                                |
| Wound            | 1 1 2                                       | 4 3 4                                |
| Body fluids      | 1 0 2                                       | 1 1 2                                |
| Other organs     | 1 0 1                                       | 4 2 4                                |
| Total (%)        | 137 (78.3)                                  | 170 (97)                              |
|                  | 99 (56.6)                                   | 48 (71.6)                             |
|                  | 170 (97)                                    | 67 (100)                              |

and chloramphenicol with 7 (10.4%).

Of 175 *E. faecalis* isolates, resistances of 9 (5%) and sensitivity of 166 (95%) isolates to vancomycin and teicoplanin were confirmed by the microdilution broth method. Of 67 *E. faecium* isolates, 51 strains (76%) were resistant to vancomycin using the disk diffusion method, but resistance of 49 strains (73%) to vancomycin was confirmed by the Microdilution Broth method. 2 strains (3%) of *E. faecium* that were determined as resistant strains by disk diffusion using the Microdilution Broth method were identified as intermediate strains. In addition, resistance of 49 (73%) and sensitivity of 18 (27%) *E. faecium* isolates to teicoplanin using disk diffusion method were confirmed by the microdilution broth method. All 9 vancomycin-resistant *E. faecalis* (VRE₉) and 49 vancomycin-resistant *Enterococcus faecium* (VRE₉₉) strains had high-level resistances to vancomycin and teicoplanin, concurrently. The MIC value among 9 VRE₉ strains and VRE₉₉ strains was ≤512, 512.

3. Presence of infective genes

The distribution of infective factors among *E. faecalis* and *E. faecium* strains are presented in Table 4 and (Fig. 2 and 3). Among the *E. faecalis* strains, the asa₁ gene was the most prevalent factor (97%), followed by the esp (78.3%) and hyl genes (56.6%); additionally, in *E. faecium* strains, the asa₁ gene had the highest prevalence (100%) and hyl gene has the lowest frequency (71.6%), followed by the esp gene (82%).

In a total of 242 Enterococci strains, the asa₁ gene was the most prevalent factor (98%), followed by the esp (79.3%) and hyl genes (60.7%). In the current study, the esp gene was found in 75% of the strains isolated from blood samples and 83% of strains isolated from wound infections.

Using statistical analysis, there was no significant difference for simultaneous occurrence of virulence genes in studied enterococci strains (p > 0.05).

DISCUSSION

In the present study, we assessed antimicrobial resistance and three infective factors in clinically isolated *E. faecalis* and *E. faecium* and further analysis was conducted to scrutinize the relationship between the presence of virulence factors and antimicrobial resistances. In this study, there was a slight discrepancy between biochemical and PCR results for species identification, which is in accordance with other studies.20,21 The main reason for this discrepancy is the similarities between *Enterococcus* species and the high phenotypic variation within individual species. PCR is a more accurate technique in comparison
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to the biochemical approach, so PCR results were preferred for the strains with discrepant identification. In this study, the esp gene was detected in 78.3% of E. faecalis and 82% of E. faecium isolates, this finding is similar to the results of other studies, which identified the esp gene in 47.1%, 23 73%,24 68.4%7 of E. faecalis, and 80%, 25 65%, 27 66%, 23 71%, 24 75%12 of E. faecium strains. However, this is in contrast to the findings of Shankar et al.28 and Channaiah et al.29 that reported the absence of esp in E. faecium. Although, as illustrated by Shankar et al.28 the esp gene was detected only in E. faecalis strains and other available studies have demonstrated a higher prevalence of the esp gene in E. faecalis,16 however, a study on the food and medical isolates indicated an increasing incidence of esp in clinical E. faecium isolates compared to E. faecalis.25

Willems demonstrated that the esp gene is a marker of the high prevalence of Enterococcus strains resistant to vancomycin in hospitalized patients,38 but according to the study of Woodford et al.,31 Sauer et al.,32 and Jahangiri et al.12 the esp gene also was identified in sensitive-vancomycin strains. In some studies, the prevalence of gene esp is reversed among VREfm and VSEfm strains. Whereas in other studies, the prevalence of gene esp was identical in VREfm and VSEfm strains. In accordance with the majority of these investigations, in the present study, the gene esp also was identified in a large number of VREfm strains (61%) compared with in VSEfm (21%). Camargo et al.33 demonstrated that esp was restricted to VREfm (56%) and not found in VSEfm. Vankerkhoven et al.10 surveyed virulence genes in eight European hospitals and found higher prevalences of esp in the clinical VREfm isolates (77%). Worth et al.34 and Sharifi et al.35 also found a higher incidence of esp in 80.5% and 71.05% in the clinical VREfm isolates, respectively. In the study by Terkuran et al.35 the esp gene was detected in 15.7% of VREfm isolates and the hyl gene in 28.6% of VREfm strains, but these genes were identified in 2.9% of Enterococcus sensitive and intermediate strains. In the study by Sauer et al.32 the esp gene was identified in 62.9% and 46.3% of VRE and VSE strains, respectively. Also, in the study by Jahangiri et al.12 esp gene was identified in 82% of clinical VREfm isolates and 53% of VSEfm isolates. The esp and hyl genes have significantly a higher prevalence among ampicillin-resistant VREfm isolates (53.7%, 37.3%) than ampicillin-susceptible VREfm isolates (19.4%, 22.4%); which is similar to the results of other studies.33-37 The high frequency of esp gene, which was shown in the present study and most of the analogous studies, could be due to the fact that strains containing this gene can obtain antibiotic resistant genes and antibiotic resistant bacteria have long term stability in the body.32,27

In the present study, the asa1 gene, (which encodes aggregation substance), was found in high frequency among E. faecalis (97%) and E. faecium strains (100%). A high incidence of this gene in E. faecalis was reported in previous studies. Results of studies on clinical E. faecium isolates are contradictory. In some studies, asa1 was not found in E. faecium but in contrast, in some studies this gene was detected in lower frequency and in our study and some other studies this gene was identified in higher prevalence among E. faecium isolates (Comerlato et al.38 and Kowalska-Krochmal et al.39), were detected it among 5%, 65% of VREfm and 2.7%, 60% of VREfm strains, respectively. Jahangiri et al.12 were not found asa1 gene in either 49 of VREfm strains or 17 of VSEfm strains. Sharifi et al.13 were detected asa1 gene in 80% of VREfm and 7.89% of VREfm strains. Hallgren et al.24 also were reported prevalence of asa1 in 79% of E. faecium strains. In studies by Huyckl & Gilmore, the asa1 gene was detected in 100% of blood isolates and 32% of non-blood isolates of Enterococcus. In studies by Elsner et al, Eaton & Gasson, and Archimbaud et al, the asa1 gene was detected in 40%-78% of clinical isolates of Enterococci.24 Hyaluronidase, coded by the chromosomal
gene **hyl**, that influence the hyaluronic acid (hyaluronate, HA). Hyaluronidase in *Enterococcus*, indicates some homology to the hyaluronidases in other bacteria such as, *Streptococcus pyogenes, Staphylococcus aureus* and *Streptococcus pneumoniae*. We found the **hyl** gene among 49.3% of VRE<sub>fm</sub> and 22.4% of VSE<sub>fm</sub> isolates, which is in accordance to findings of Rice et al. who detected the **hyl** gene among 71% of the United Kingdom VRE<sub>fm</sub> isolates. But it was in contrast to the study by Jahangiri et al. which detected **hyl** gene among 80% of VSE<sub>fm</sub> and in 28.5% of VRE<sub>fm</sub> isolates.

The reasons for the diversity in frequencies of **hyl** and **asa**<sub>1</sub> can be as follows; *Enterococcus* strains are genetically different from each other based on their geographical origins. Moreover, the media which were used as sampling sources were varied between studies. In other words, some studies took their samples from blood, while others used urine, foods, or sewage. As demonstrated by Billström et al. and Wardal et al. the **esp** and **hyl** genes are linked with ampicillin and ciprofloxacin-resistant *enterococci*, particularly in CC<sub>17</sub> which is an especially virulent, hospital adapted clone found globally. In the present study, it was shown that the **esp** virulence gene has a significant association with ciprofloxacin (p=0.001), erythromycin (p=0.001) and tetracycline (p=0.001) susceptibility patterns in *E. faecium* and with chloramphenicol (p=0.001) in *E. faecalis* strains; the **hyl** with teicoplanin (p=0.001) and vancomycin (p=0.001) in *E. faecium* strains; and also **asa**<sub>1</sub> with vancomycin (p=0.001) in *E. faecium* and with ampicillin (p=0.001) and chloramphenicol (p=0.001) in *E. faecalis* strains (Table 5).

The correlation between infective genes and antibiotic resistance in *Enterococcus* may vary from country to country. Based on the study by Hanna Billström et al. a significant relationship between imipenem, ampicillin, and ciprofloxacin resistance pattern and the **esp**<sub>fm</sub> gene was found. Resistance to ciprofloxacin, imipenem, ampicillin antibiotics and the prevalence of the **esp** and **hyl** genes in these isolates were reported 90%, 80%, 77%, 56% and 4%, respectively. In the Study by Baylan et al. (Turkey, 2008) on *E. faecalis* strains, a significant association between the carriage **asa**<sub>1</sub> gene and ciprofloxacin, norfloxacin and levofloxacin resistance pattern and between the **esp** gene and doxycycline resistance pattern were observed; in addition, a significant association between the **hyl** gene and nitrofurantoin resistance pattern in *E. faecium* strains was indicated. In the other study by Jankoska et al. there was no significant relationship between virulence genes and antibiotic resistance patterns; the **esp** gene was detected in 76% of isolates and all strains were susceptible to vancomycin and nitrofurantoin, 24%, 34%, and 28% of strains were resistant to ampicillin, ciprofloxacin and ceftriaxone, respectively.

According to the results of studies by Jahangiri et al. and Duprè, it was found that most of **esp**-positive isolates were resistant to more than 3 antibiotics. Lund et al. demonstrated that the existence of a strong correlation between the carriage of **esp** gene and antimicrobial resistance could be due to the higher conjugation frequencies in strains carrying the **esp** gene compared with strains lacking this gene. Sharifi et al. showed that *E. faecium* strains carrying the **esp** gene were resistant to more than 90% of the tested antibiotics and 64% of them were resistant to vancomycin. Considering these results, it seems that the **esp** gene facilitates *E. faecium* isolates ability to acquire antibiotic resistance genes. Van Wamel et al. showed that the expression level of the **esp** gene vary constantly between *E. faecium* strains depending on growth conditions and it is associated with both the initial connection and biofilm formation. Due to increasing resistance rates of enterococci to most common antibiotics, strict infection control measures are required. Antibiotic susceptibility testing is recommended for all patients before treatment for rational antibiotic use. There is a significant relationship between virulence genes and antibiotic resistance patterns. The virulence factors involve conjugative transfer of antibiotic resistance genes among enterococci strains and other species especially as the transfer of vancomycin resistance to *staphylococcus aureus* strains may occur.

### Table 5: Statistical analysis for determining possible relationship between antibiotic resistance pattern and **esp**, **hyl**, **asa**<sub>1</sub> virulence genes in *E. faecium* and *E. faecalis* strains

| Antimicrobial agents | **E. faecium** | **E. faecalis** |
|---------------------|---------------|----------------|
|                     | esp | hyl | **asa**<sub>1</sub> | esp | hyl | **asa**<sub>1</sub> |
| CIP                 | p=0.001 | p=0.037 | p > 0.05 | p > 0.05 | p > 0.05 | p > 0.05 |
| E                   | p=0.001 | p=0.048 | p > 0.05 | p > 0.05 | p > 0.05 | p > 0.05 |
| TEC                 | p=0.048 | p=0.001 | p=0.002 | p > 0.05 | p > 0.05 | p > 0.05 |
| VAN                 | p=0.048 | p=0.001 | p=0.001 | p=0.048 | p > 0.05 | p > 0.05 |
| T                   | p=0.001 | p=0.020 | p > 0.05 | p > 0.05 | p > 0.05 | p > 0.05 |
| A                   | p=0.037 | p=0.020 | p > 0.05 | p > 0.05 | p > 0.05 | p=0.001 |
| C                   | p > 0.05 | p > 0.05 | p > 0.05 | p=0.001 | p > 0.05 | p=0.001 |
| NOR                 | p > 0.05 | p > 0.05 | p > 0.05 | p=0.003 | p > 0.05 | p > 0.05 |

CIP: ciprofloxacin, E: erythromycin, TEC: teicoplanin, VAN: vancomycin, T: tetracycline, A: ampicillin, C: chloramphenicol, NOR: norfloxacin. p: probability value based on Chi-Square test.
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CONFLICT OF INTEREST STATEMENT

None declared.

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