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Virulence Factors of Clinical and Fecal Isolates of *Enterococci* Species

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Abstract

***Enterococci* species are known commensals of the gastrointestinal flora; however, in recent years, they have emerged as important nosocomial pathogens that possess many virulence factors that are attributed to the pathogenesis of diseases caused by them. The study evaluated and compared the virulence factors of *Enterococci* isolated from fecal and clinical samples. From the obtained isolates, the clinical enterococcal isolates produced 35%, 20%, and 50%, and fecal isolates produced 23%, 13%, and 13% gelatinase, hemolysin, and biofilm, respectively. Biofilm production determined by the Congo Red agar, tube, and microtiter plate methods was 23%, 39%, and 49%, respectively. The sensitivity of the Congo Red agar and tube method compared to the microtiter plate method was 27% and 46%, respectively, whereas the specificity of both tests was 79%. This study showed that biofilm production plays a significant role in the pathogenesis of diseases caused by *Enterococci*. Detection of biofilm production using the microtiter plate method is more sensitive and specific than the Congo Red agar and tube method.**

Keywords: Biofilms, Enterococci, Hemolysin, Gelatinase, Virulence Factors

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INTRODUCTION

Enterococci, commensals of the genital tract, oral cavity, and gastrointestinal tract, have recently emerged as nosocomial pathogens that cause serious infections such as bloodstream infections (the incidence of which is steadily increasing), urinary tract infections (UTI), catheter-associated UTI (CAUTI), and intra-abdominal or intrapelvic abscesses.[1-3] Among the *Enterococcus* species, *E. faecalis* is responsible for human enterococcal infections in 80–90% of cases, followed by *E. faecium*.[3] *Enterococci* are intrinsically resistant to antibiotics due to the acquisition of genetic sequences responsible for drug resistance in other bacteria by transferring plasmids, transposons, or chromosome mutations.[4-6]

In addition to studies analyzing the trend of increased drug resistance in these organisms, several virulence factors have been studied. A few hemolysins, gelatinase, can form biofilms (enterococcal surface proteins). In contrast, other putative virulence factors, such as hyaluronidase, are not considered to be the disease-causing capability of *Enterococcus* strains.[2] Several studies have reported the prevalence of *Enterococci* in India.[7] However, few studies have focused on the prevalence of virulence factors in *Enterococci*. This study evaluated and compared the virulence factors of *Enterococci* isolated from fecal and clinical samples.

MATERIALS AND METHODS

The study was conducted in the Department of Microbiology, R L Jalappa Hospital, and Research Center for over one year. Seventy-five clinical isolates of *Enterococcus* species were included in this study. The isolates were obtained from blood, sterile body fluids (cerebrospinal fluid, peritoneal fluid, and pleural fluid), urine, and pus/wound swabs. Routine bacteriological methods were used to isolate and identify *Enterococcus* species.[8,9]

Thirty isolates of *Enterococcus* species were collected from stool samples to compare virulence factors. The institutional ethics committee approved this study. The isolated clinical *Enterococcus* strains and commensal strains (from stool samples) of *Enterococcus* species were subjected to phenotypic methods to detect virulence factors. Hemolysin was detected using brain-heart infusion agar supplemented with 5% human blood. Gelatinase production was detected using peptone yeast extract agar containing 30 g/L gelatin. Biofilm production was detected using Congo Red agar, tube, and microtiter plate methods.[2,3,10,11]

RESULTS

Seventy-five *Enterococcus* clinical isolates and 30 fecal isolates were tested for virulence

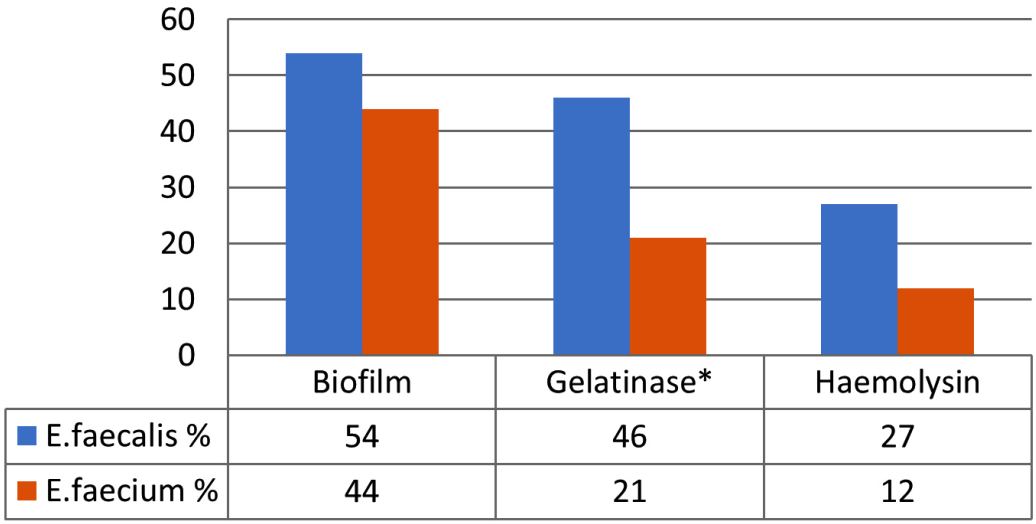

Figure 1. Virulence factors in *Enterococcus* species of clinical isolates

factors. Of the 75 clinical *Enterococci*, 41 (55%) were *E. faecalis* and 34 (45%) were *E. faecium*. In contrast, among the 30 fecal isolates of *Enterococci*, 60% were *E. faecium* and 40% were *E. faecalis*.

Table 1 shows that out of 75 strains of *Enterococci* isolated from clinical samples, 26 (35%), 15 (20%), and 35 (50%) were gelatinase, hemolysin, and biofilm producers, respectively. In contrast, out of 30 fecal isolates of *Enterococci*, 7 (23%), 4 (13.3%), and 4 (13.3%) were gelatinase, hemolysin, and biofilm producers, respectively. Biofilm production was observed significantly more frequently in clinical isolates ($p < 0.05$).

Figure 1 shows the virulence factors in *Enterococcus* species of the clinical isolates. In total, 54%, 46%, and 27% of isolates of *E. faecalis* were biofilm, gelatinase, and hemolysin producers. Among *E. faecium* isolates, 44%, 21%, and 12% were biofilm, gelatinase, and hemolysin producers, respectively. (* Gelatinase production: statistically significant, $p = 0.0281$)

Figure 2 shows the biofilm detection using different methods. The microtiter plate method (49%) was more sensitive than the Congo Red agar (23%) and tube methods (39%). The sensitivity of the Congo Red agar and tube method compared to the microtiter plate method was 27% and 46%, respectively, whereas the specificity of both tests was 79%. Among the virulence factors, higher biofilm production was observed in *Enterococci* isolated from UTI 7 (70%), CAUTI 4 (50%), sepsis 5 (45%), postoperative wound infection 8 (44%), and diabetic wound infection 3 (43%) (Table 2).

Table 1. Gelatinase, hemolysin and biofilm production in pathogenic and fecal isolates

Virulence factors	Clinical isolates n = 75 (%)	Fecal isolates n = 30 (%)	P value
Gelatinase	26 (35%)	7 (23%)	0.2593
Hemolysin	15 (20%)	4 (13.3%)	0.4228
Biofilm	35 (46.6%)	4 (13.3%)	0.0303 (<0.05) (statistically significant)

Table 2. Virulence factors and associated clinical conditions

Clinical conditions (N)	Biofilm production	Gelatinase production	Hemolysin production
Urinary tract infection (UTI) (10)	7 (70%)	3 (30%)	-
Catheter-associated UTI (8)	4 (50%)	2 (25%)	2 (25%)
Sepsis (11)	5 (45%)	2 (18%)	3 (27%)
Post-op wound infection (18)	8 (44%)	7 (39%)	3 (17%)
Diabetic wound infection (7)	3 (43%)	2 (29%)	3 (43%)
Wound infection (12)	5 (42%)	8 (67%)	3 (25%)
Perforation (3)	1 (33%)	-	1 (33%)

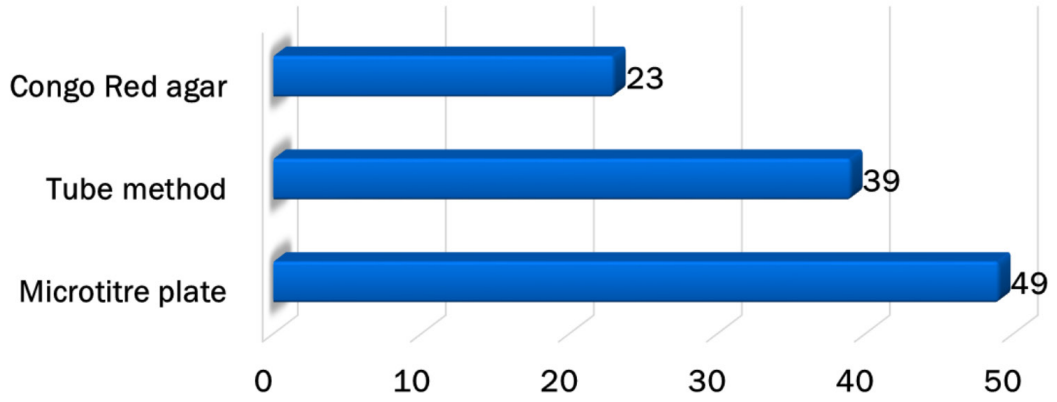

Figure 2. Biofilm detection by different methods

DISCUSSION

Enterococci are emerging as important nosocomial pathogens due to their ability to acquire and spread genes responsible for antibiotic resistance. However, the role of other virulence factors of *Enterococci* in causing the disease cannot be neglected.[2]

Enterococci are well-adapted to areas with low redox potential, such as the oral cavity, gut, and genitourinary tract.[9,12] *Enterococci* first adhere to specific host tissues, invade, and exert their pathogenic effects. In unfavorable environments, the expression of various enterococcal traits ultimately contributes to virulence.[13]

With the emergence of new virulence factors in *Enterococci*, they evolved like other pathogenic organisms. Antibiotic resistance has been the best studied in *E. faecium*. In contrast, virulence traits have been studied in *E. faecalis*. Increased virulence is assumed to be directly associated with increased antibiotic resistance. However, cost–benefit analyses have shown that virulence and antibiotic resistance are two completely different aspects of bacterial cells; hence, increased virulence may or may not be associated with increased antibiotic resistance in bacterial cells.[13-15] Therefore, intrinsic virulence and antibiotic resistance play roles in the pathogenesis of diseases caused by *Enterococci*; however, in a complementary way.[7,16]

In our study, of the 75 clinical *Enterococci* isolates, the predominant species isolated was *E. faecalis* (55%), followed by *E. faecium* (45%). The distribution ratio of *E. faecalis* to *E. faecium* is similar to that reported in other studies.[17-20]

We studied biofilm formation, hemolysin, and gelatinase production in fecal and clinical isolates. We observed no significant differences in the production of hemolysin and gelatinase between the fecal and clinical isolates. However, for biofilm production, a statistically significant difference ($P < 0.05$) was observed between the clinical and fecal isolates, similar to studies by Upadhyaya et al. and Jett et al.[3,11] Nosocomial strains are known to develop different mechanisms of colonization and cause infection. One of these mechanisms is the production of biofilms that facilitate both surface adherence and invasion of host cells by the organism causing the infection. Our results concord with those of Ira et al., who reported that gelatinase production was statistically significant among clinical isolates.[2]

Gelatinase production was considerably higher than biofilm and hemolysin production in *E. faecalis* isolates (46%) than in *E. faecium* (21%) ($p = 0.0281$). Thus, biofilm production is responsible for more infections by *E. faecalis* than by *E. faecium*. Gelatinase and hemolysin production help *Enterococci* spread infection, thus increasing the severity of the infection. The production of gelatinase and hemolysin helps acquire and meet *Enterococci's* nutritional needs. Our results are similar to those reported by Fernandes et al. and Sood et al.[1,19]

In this study, biofilm detection using the microtiter plate method was more sensitive (49%) than the Congo Red agar (23%) and tube methods (39%). In contrast, a study by Ruchi et al.[20] showed that the Congo Red agar method was more sensitive (40%) for biofilm detection than the tube method (37%) and microtiter plate method (27%).

The microtiter plate method is preferred because it is easy to perform, cost-effective, and assesses the biofilm-forming capacity of an isolate both qualitatively and quantitatively. The subjective error in interpreting biofilm production findings was overcome using an enzyme-linked immunosorbent assay reader. Other methods, such as epifluorescence microscopy and microscopic biofilm formation assays, have been used to study the biofilm-forming abilities of bacteria. The Congo Red agar method is a reproducible and rapid technique that is sensitive to the added advantage of test colonies on the medium remaining viable. Moreover, subjective errors during reporting were observed more frequently with the tube method than with the Congo Red agar method.[2,21]

In this study, biofilm production was higher in clinical isolates than in fecal isolates. This likely plays a key role in the pathogenesis of diseases caused by *Enterococci*. These results are similar to those reported by Fernandes et al., Hemalatha et al., and Marothi et al.[1,16,21]

Enterococci have been associated with biofilm production on various types of indwelling devices, such as prosthetic heart valves, urinary

catheters, and artificial hip prostheses, and this capability to produce biofilms is considered an important virulence factor of this organism.[2]

CONCLUSION

This study showed that biofilm production plays a significant role in the pathogenesis of diseases caused by *Enterococci*. Detection of biofilm production using the microtiter plate method is more sensitive and specific than the Congo Red agar and tube method. Clinical isolates from patients with UTI, CAUTIs, and septicemia showed higher rates of biofilm production than fecal isolates. Mechanisms or interventions to stop biofilm production could have added advantages in managing patients suffering from nosocomial infections caused by multidrug-resistant *Enterococci*.

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None.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

SK, UPCR and PMB conceptualized and designed the work. UPCR, AN, SM and SK performed data analysis. AN, SM and SK performed data interpretation. SK and SM wrote the manuscript. PMB supervised the study. AN approved the final manuscript for publication.

FUNDING

None.

DATA AVAILABILITY

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

This study was approved by the Institutional Ethics Committee, Sri Devaraj URS Medical College, Tamaka, Kolar, India, with reference number DMC/KLR/UDOME/IEC-CER/117/2014-15.

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