



EVALUATION OF BIOFILM FORMING CAPACITY OF ENTEROCOCCI BY TISSUE CULTURE PLATE METHOD

Medical Microbiology

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ABSTRACT

Health care associated infections have become a major concern among the health care set-up as they are highly complicated diseases to manage and treat. Majority of them are caused by multidrug resistant microorganisms like Methicillin Resistant *Staphylococcus aureus* (MRSA), Vancomycin Resistant Enterococci (VRE), and multidrug resistant *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. Bacteria causing nosocomial infections have the capability to resist environmental hardships and remain viable for longer duration on inanimate objects, one of the main virulence factor giving the survival advantage to these bacteria is the capacity to form biofilm. Enterococci has the ability to form biofilm and it causes severe device associated health care infections like CAUTI and VAP. Identifying the process of biofilm formation in Enterococci and their role in pathogenesis helps to control hospital acquired infections & to overcome the antibiotic resistance.

KEYWORDS

Health care associated infections, biofilm and Enterococci.

INTRODUCTION

Enterococci are found as normal commensal bacteria in human oral cavity, gastro-intestinal tract, female genital tract. They have been involved in causing many infections like urinary tract infection, pelvic-inflammatory infection in females, intra-abdominal infection, skin and soft tissue infections like cellulitis, bacteremia, bacterial endocarditis, meningitis and spontaneous bacterial peritonitis, hospital acquired infections like CAUTI and VAP.(1). *Enterococcus faecalis* and *Enterococcus faecium* are the two most commonly isolated species of Enterococci. (2)

Enterococci are the oval shaped Gram-positive cocci, they are non-capsulated, non-spore forming and non-motile bacteria (3). They readily grow on nutrient agar and medium with high salt content. On blood agar it produces hemolysis sometimes. They ferment sugars like mannitol, arabinose and others by producing acid with no gas. They are capable of withstanding very harsh and unfavorable conditions by producing many virulence factors. (4)

These virulence factors are also involved in causing severe infections in the susceptible host. The virulence factors produced by Enterococci are cytolysin, hyaluronidase, gelatinase, hemolysin, aggregation factor and Enterococcal surface protein (5). The important virulence property of Enterococci is the capacity to produce biofilm, which is highly pathogenic in nature. The biofilm formation is involved in causing many life-threatening nosocomial infections.

Infections associated with biofilm are initially confined to a particular location and detached biofilm cells enter bloodstream and cause infections. Enterococcal surface protein encoded by *esp* gene is associated with increased virulence, colonization in urinary tract and form biofilm formation. Aggregation substance encoded by *asa1* gene facilitates the conjugative transfer of sex pheromone gene containing plasmids which enhances adherence to renal tubular cells. Gelatinase encoded by *GeE* gene damages the host tissue facilitating bacterial migration and spread colonization.(6)

The biofilm formation in Enterococci is mediated by various mechanisms like quorum sensing system and auto-inducing pheromones. The cells in biofilm are also involved in horizontal transfer of antibiotic-resistance genes to another bacterium within the biofilm.

Severe Enterococcal infections are mainly due to the development of resistance towards antibiotics like β -lactams, cephalosporins, aminoglycosides, glycopeptides and other clinically used antibiotics (7). The mechanism of developing resistance in Enterococci is either due to intrinsic or acquired through horizontal transfer of antibiotic-resistance gene.

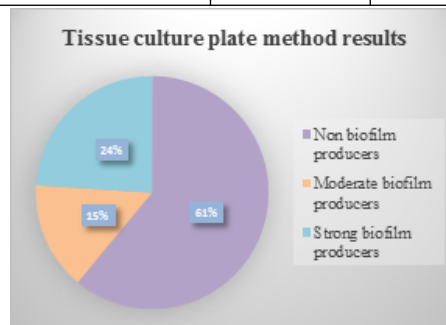
Case Study

In the present prospective based hospital study, a total of 100 clinical

isolates of Enterococci isolated from urine samples were subjected to biofilm detection by tissue culture plate method. Out of 100 isolates, 61(61%) were found to be non-biofilm producers and 39 (39%) were found to be biofilm producers. Among the 39 biofilm producers, 15 (15%) were moderate biofilm producers and 24 (24%) were strong biofilm producers. The study was carried over a period of 6 months in the department of microbiology, JSS medical college, Mysuru.

Table 1: Tissue culture plate method test results

TCP results	No. of isolates	In percentage
Non biofilm producers	61	61%
Moderate biofilm producers	15	15%
Strong biofilm producers	24	24%
Total	100	100 %



Graph 1: Tissue culture plate method results

CONCLUSIONS

Enterococci are the highly virulent bacteria which causes both community acquired and hospital acquired infections by producing many virulence factors like hemolysin, cytolysin, gelatinase and biofilm formation. The cells in biofilm are highly virulent and causes severe infections among the chronic patients, in which majority of them are multidrug resistant. Acquiring resistance to antibiotics is another important property of the biofilm producing Enterococci. The cells in the biofilm are not only involved in acquiring resistance to antibiotics but also horizontally transfers the antibiotic-resistant genes to another bacterium within the biofilm. This study found that there was a significant amount of biofilm production in Enterococci by tissue culture plate method.

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