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Antimicrobial Susceptibility of Biofilm Producing Enterococcus Faecalis Isolated From Clinical Samples

KEYWORDS

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ABSTRACT *The aim of the study is to evaluate Antimicrobial Susceptibility of Biofilm producing Enterococcus faecalis isolated from Clinical Samples. For detection of Antimicrobial Susceptibility 198 clinical isolates of E. faecalis were tested for antibiotic susceptibility by Kirby-Bauer disc diffusion method (CLSI, 2007).The isolates from clinical samples were tested for sensitivity to clinically attainable levels of six antibiotics viz. Vancomycin, Ampicillin, Chloramphenicol, Rifampicin, Gentamicin and Teicoplanin. Out of the 198 Enterococcus faecalis 48.28% resistant to Rifampicin (30µg/ml), 50.32% resistant to Gentamicin (10µg/ml),45.12% resistant to Chloramphenicol (30µg/ml) and 63.19% sensitive to Vancomycin (10µg/ml), 55.26% sensitive to Teicoplanin (30µg/ml), 52.84% sensitive to Ampicillin (10µg/ml). During observation it was seen that E. faecalis showed maximum sensitivity to Vancomycin and maximum resistance to Gentamicin*

E. faecalis is the most common species which causes severe infections in humans (Iwen et al., 1997; Top et al., 2008) such as bacteraemia, endocarditis and urinary tract infections (Hendrickx et al., 2007). There are two ways by which Enterococci can cause infections. The first type originate from the patient's native flora. These Enterococci are unlikely to possess acquired antibiotic resistance, but possess intrinsic resistance that is normal for the species. The second type of infections are caused by bacteria that often possess acquired resistance to antibiotics, in addition to the intrinsic, and are capable of nosocomial transmission. Spread of Enterococci between patients probably goes via hands of health care providers and medical devices, while spread within hospitals is caused by patients with prolonged intestinal colonization (Huycke et al., 1998).

Clinical infections can involve almost any anatomic site and may be life-threatening during bacteriemia and endocarditis (Murray, 1990; Lu et al., 2002). Resistance to Vancomycin in Enterococci more than doubles the odds of dying during bloodstream infections by Vancomycin Resistance Enterococci i.e. VRE (Diaz Grandos et al., 2005). In a study, Enterococci were found to be the only Gram-positive pathogen independently associated with high risk of death in bloodstream infections (Weinstein et al., 1983). Enterococcal bacteriemia leads to death in 12 - 68% of the cases and Enterococcal sepsis causes 4-50% of deaths (Jett et al., 1994).

In 1986, cases of Glycopeptides Resistant Enterococci were reported in Europe and from all over the world. Infections caused by Vancomycin Resistant Enterococci (VRE) in the US increased from 0 to 28.5% between 1989-2003. In 1990s VRE had become the second most common nosocomial pathogen and were endemic in many hospitals around the US. Further colonization of VRE in hospitalized patients also increased rapidly at this time to reach the present levels. In Europe, the prevalence rates in hospitals remained much lower until 2000, after which it started to increase. This was found to be due to much higher use of Vancomycin in U.S. hospitals than in European hospitals as reported in a comparative study between the U.S. and European countries with similar number of inhabitants (Top et al., 2008).

Antibiotic Resistance

Antibiotic resistance is more frequent in hospital environment than in the community. It is often caused by antibiotic pressure in the environment of the bacteria. Main mechanisms for antibiotic resistance are inactivation of the drug, prevention of the drug to reach its target site, reduction of the target susceptibility and acquisition of a new less sensitive target (Berger-Bachi, 2002). Enterococci harbor both intrinsic and acquired drug resistance. A new drug Tigecycline with Anti-Enterococci activity has recently been found that can be used to treat infections caused by Enterococci (Pankey, 2005).

E. faecalis resistance to Quinopristin-Dalfopristin, community reservoir in US and Europe has emerged due to the widespread use of the analogue Virginiamycin as a growth promoter (Acar et al., 2000). Acquired drug resistance is often due to mutations and exchange or acquisition of genetic mobile elements such as transposons and plasmids.

The genetic mobile elements often contain genes for virulence factors other than resistance to antibiotics. Enterococci can transfer these elements to both Gram negative and Gram positive bacteria, which make them even more potent. E. faecium, E. hirae and E. faecalis are known to acquire high level resistance to Ampicillin by over production of Penicillin binding proteins or in some cases production of β-lactamase (Top et al., 2008).

The nosocomial pathogen E. faecalis is a normal resident of the intestinal tract of many mammals including humans and is readily isolated from many other environments. It can form biofilms on biotic and abiotic surfaces and Enterococcal biofilms likely play a role in virulence, persistence and antibiotic prop-

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erty of this organism.

Materials and Methods:-

Bacterial Strains:- The clinical isolates of Enterococcus spp, were isolated from blood, urine and infected devices taken from S.M.S Medical College and Hospital, Jaipur. The 198 clinical isolates of E. faecalis were then assessed for pathogenicity and antibiotic susceptibility of this bacterium. The control strain used in this method was E. faecalis ATCC 47077 ($O.D_{490}$ = 0.20) which was obtained from Himedia, Mumbai. (Table 1)

Table - 1
Clinical samples from which E. faecalis **were isolated**

Samples	E. faecalis
Catheter tips (57)	53(26.7%)*
Urine (55)	47(23.7%)
Blood (45)	39(19.7%)
Pus (25)	20(10.1%)
Ear swabs (21)	20(10.1%)
Wounds (22)	19(9.5%)
Total	198

*Maximum (26.7%) isolates of E. feacalis were isolated from catheter tips.

Method for detecting Antimicrobial Susceptibility :-
The biofilm forming E. faecalis were then tested for antibiotic susceptibility by **Kirby-Bauer disc diffusion method** (CLSI, 2007) as follows:

The isolates from clinical samples were tested for sensitivity to clinically attainable levels of six antibiotics viz. Vancomycin, Ampicillin, Chloramphenicol, Rifampicin, Gentamicin and Teicoplanin.

The Kirby-Bauer method is based on the inhibition of bacterial growth measured under standard conditions.

- The organism to be tested was grown to a specific turbidity in a standard liquid medium (0.5 McFarland turbidity standards approx. 1.0×10^8 CFU/ml) and compared visually with $BaSO_4$ 0.5 McFarland standard.

- Inocula from this culture were spread across the surface of Mueller Hinton agar (MHA) plates to give confluent growth.

- Paper discs containing specific concentrations of each selected antibiotic were placed on the agar surface.

- After incubation, the diameter of the zone of growth inhibition was measured and scored according to the size of the zone.

- For each antibiotic the sensitivity was indicated as sensitive or resistant.

The size of the zone of inhibition is directly proportional to the sensitivity of the organism to the antibiotic.

* Sensitive zone of inhibition >3mm in diameter.

* Resistant zone of inhibition< 3mm in diameter.

Results :-

1) Antimicrobial Susceptibility testing

This test was carried out by disc diffusion method. Antibiotic resistance and sensitivity pattern of E. faecalis is as follows:

- Resistant to Rifampicin (30µg/ml) – 48.28%
- Resistant to Gentamicin (10µg/ml) – 50.32%
- Resistant to Chloramphenicol (30µg/ml) – 45.12%
- Sensitive to Vancomycin (10µg/ml) – 63.19%
- Sensitive to Teicoplanin(30µg/ml) - 55.26%
- Sensitive to Ampicillin (10µg/ml) - 52.84%

These results are shown in Table 2, Fig. 1 and 2.

Table - 2
Antibiotic Sensitivity/Resistance as shown by Isolates of E. faecalis

No. of isolates ----- 198			
Antibiotic Sensitivity	Vancomycin* (10µg/ml)	Teicoplanin (30µg/ml)	Ampicillin (10µg/ml)
	63.19%	55.26%	52.84%
Antibiotic Resistance	Gentamicin* (10µg/ml)	Rifampicin (30µg/ml)	Chloramphenicol (30µg/ml)
	50.32%	48.28%	45.12%

***** E. faecalis **showed maximum resistance to Gentamicin and maximum sensitivity to Vancomycin.**

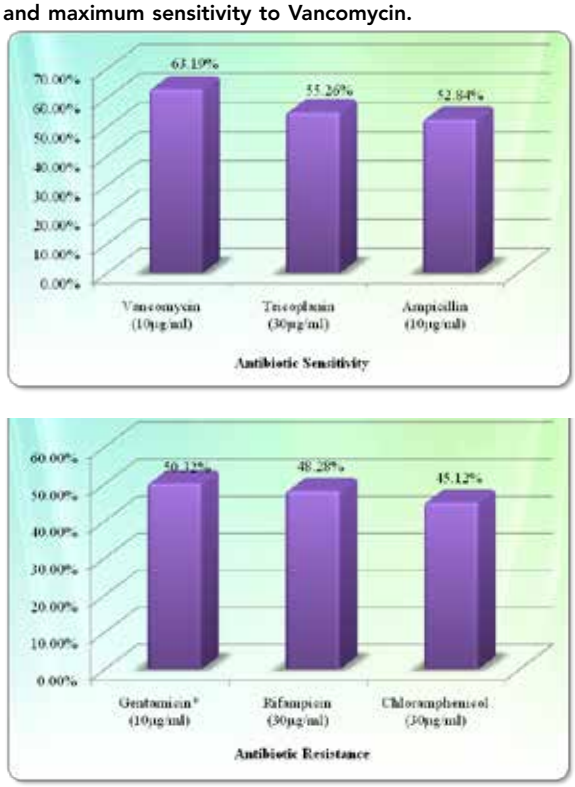

Figure 2 : Antibiotic Resistance as shown by isolates of E. faecalis

Discussion:-

In the present study, an attempt was made to assess the resistance of E. faecalis to various antibiotics. For this, the susceptibility test was carried out by Disc diffusion method as recommended by the National Committee for Clinical Laboratory Standards (2000). The isolates showed high resistance to the antibiotics tested: a maximum of 50.32% were resistant to Gentamicin (Table 2; Fig. 2). Recently,

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Bose et al. (2012) , Chandrakanth et al.(2012), Shafiyabi et al.(2013), and Jada and Kumar (2013) also reported Enterococci to exhibit resistance to Gentamicin. On the other hand, Acharya et al. (2003) and Sreeja et al. (2012) found the isolates to be 62% and 55.2% susceptible to this antibiotic respectively.

Resistance to Rifampicin was shown by 48.28% isolates in the present study (Table 2).

Sharifi et al. (2013) found 86.2% isolates to be resistant to the same drug. Whereas, Chandrakanth et al. (2012) observed that 61.84% isolates were susceptible.

E. faecalis isolates (45.12%) showed resistance to Chloramphenicol (Table 2). This is in accordance with Patidar et al. (2012) and Gopinath and Prakash (2013). On the contrary, Acharya et al. (2003) and Willems et al. (2006) found the isolates to be susceptible to Chloramphenicol.

Further, 63.19% isolates were found to be sensitive to Vancomycin, 55.26% to Teicoplanin and 52.84% to Ampicillin (Table 2).

Acharya et al. (2003), Butt et al. (2004), Chayakul et al. (2007), Chaudhary et al. (2007) and Patidar et al. (2012) too reported E. faecalis to be susceptible to Vancomycin. However, Sepandj et al. (2007), Chandrakanth et al. (2012), Gopinath and Prakash (2013) and Jada and Kumar (2013) found the isolates to be resistant to Vancomycin.

Sensitivity of E. faecalis for Teicoplanin has also been observed by other researchers. According to Chaudhary et al. (2007) and Agarwal et al. (2009) 88% and 100% isolates were found to be sensitive respectively. Sreeja et al. (2012) found 65.7% isolates to be sensitive and 34.2% to be resistant to Teicoplanin. Sharifi et al. (2013) reported only 18.6% isolates to be resistant to this drug.

Susceptibility of E. faecalis to Ampicillin is supported by the studies of Acharya et al. (2003), Butt et al. (2004) and Chandrakanth et al. (2012).

Sreeja et al. (2012) reported 52.6% isolates to be sensitive and 47.3% to be resistant to Ampicillin. Sharifi et al. (2013) found 28.2% isolates to be resistant to this antibiotic.

E. faecalis has also been reported to be resistant to some other antibiotics such as Penicillin (Sreeja et al.,2012; Jada and Kumar, 2013; Sharifi et al., 2013 and Shafiyabi et al., 2013), Tetracycline (Bose et al., 2012; Patidar et al., 2012 and Shafiyabi et al., 2013), Streptomycin (Gopinath and Prakash, 2013), Erythromycin (Patidar et al., 2012; Jada and Kumar, 2013 and Shafiyabi et al., 2013), Cephalothin and Ofloxacin (Trivedi et al., 2011).

Patel et al. (2011) found the same isolate to be resistant to Cloxacillin, Lincomycin, Cephalexin, Bacitracin, Roxythromycin and Levofloxacin.

There are reports that indicate sensitivity of E. faecalis towards certain antibiotics such as Ciprofloxacin (Acharya et al., 2003; Willems et al., 2006; Savas et al., 2006; Chandrakanth et al., 2012 and Sreeja et al., 2012), Amikacin (Savas et al., 2006; Abdallah et al., 2011), Aoxicillin (Pinherio et al., 2006; Patidar et al., 2012), Novobiocin, Spectinomycin and Doxycycline (Chaudhary et al.,2007), Streptomycin and Tobramycin (Chandrakanth et al., 2012) and Levofloxacin (Bose et al., 2012).

Conclusion:- Monitoring the antibiotic resistance, antibiotic use and studies on the dissemination of antibiotic resistance in humans is essential to obtain consistent and reliable data on the epidemiology of resistance and susceptibility of Enterococcal isolates from humans for the treatment of Enterococcal infections. There is an urgent need for the development of novel antimicrobial agents against the highly resistant E. faecalis. It may therefore be concluded that E. faecalis showed 63.19% sensitivity to Vancomycin and 50.32% resistance to Gentamicin

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