DETECTION OF BETA LACTAMASE FROM THE CLINICAL ISOLATES OF ENTEROCOCCUS SPECIES

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Abstract

β-lactamase is an enzyme that deactivates β-lactam antibiotics such as penicillins and cephalosporins. The b-lactam enzyme hydrolyzes (ie, breaks open) the b-lactam ring, destroying the antibacterial properties of the molecule. A sum of 20 clinical isolates of Enterococcus isolates were subjected to antibiotic susceptibility pattern followed by beta lactamase production by tube method. In this method, we have observed 4/20 (20%) of our Enterococcus isolates were found to be β-lactamse positive. As enteroccocus species are frequently isolated from different clinical specimen and the rate of drug sensitivity to different antibiotics have been reduced a lot due to various mechanisms. It is very important role in the selection of drugs and better treatement outcomes.

Key words: *Enterococcus, antibiotic susceptibility pattern, beta lactamase*

INTRODUCTION:

β-lactamase is an enzyme that deactivates β-lactam antibiotics such as penicillins and cephalosporins. The b-lactam enzyme hydrolyzes (ie, breaks open) the b-lactam ring, destroying the antibacterial properties of the molecule. [1] Enterococcus causes significant infections including abdominal sepsis,urinary tract infection , bacterimias and endocarditis. High level gentamicin resistance has been invented ina isolates of both Enterococcus faeciumand faecalis. Enterococcus faecalis and faecium are predominantly found to be causing infections as per the clinical isolates.[2] The mechanism of resistance among the streptococcus is even more greater in the s.faecium is still incompletely understood. Hyper susceptibility to beta lactamase in these strains can be due toabsence or alternation in one or more cell membrane. The increasing importance to the enterococcus in the nosocomial infections is due to the many inheritance and acquired resistance found in these organisms.[3] They generally have resistance towards number of antimicrobial agents such as tetracycline,clindamycin, erythromycin, high level of penicillin, high level of aminoglycosides, and more recently vancomycin. [4]

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The recent studies show that the first isolates of beta lactamase was found in enterococcus faecalis [5]. Since other enterococcal infections have been isolated in the various regions as nosocomial pathogens , the gene coding for beta lactamase is found in both Staphylococcus aureus and Enterococcus faecalis are found on plasmids. [6,7,8]

MATERIALS AND METHODS:

Clinical isolates:

A total of 20 different non-repetitive clinic isolates of Enterococci were collected from different clinical specimens were included in this study. These isolates were identified by standard biochemical parameters as described by elsewhere. Isolates were preserved in semi-solid brain heart infusion medium and stored at 4ºC until further use.

Antimicrobial susceptibility test:

Antibiotic susceptibility test was determined for these strains to routinely used antibiotics such as ampicillin (10µ), vancomycin (30µ), teicoplanin (30µ), erythromycin (15µ), ciprofloxacin (5µ), amikacin (200µ), gentamycin (10µ), tetracycline (30µ) and linezolid (30µ) (Hi Media, Mumbai) by kirby-bauer disc diffusion method.[9]

Detection of β-lactamse by tube method:

The sum of 20 samples was collected from the out patient department of Saveethadental college. Benzoyl penicillin, 6 mg/dL in 0.1 in phosphate bufferpH 6.0, is distributed in 0.1 mL quantities in the titre tube. The bacterial growth suspension in agar is put to this solution until turbidity turns heavily. They are held in the 30-60 minutes. Followed by this 20 microlitre volume of 1% starch is added followed by iodine in aqueous potassium iodide. Beta lactamase activity is demonstrated by the decolorisation of iodine within 5 mins. Heavily inoculated can give false negative results. [10]

RESULTS:

Sample wise distribution of clinical isolates of Enterococci:

Of the 20 clinical isolates of Enterococci, 12/20 (60%) were obtained from urine, 4/20 (20%) were from blood, 2/20 (10%) and 2/20 (10%) were from stool samples and wound swabs respectively. Figure 1 depicts sample wise distribution of clinical isolates of Enterococci.

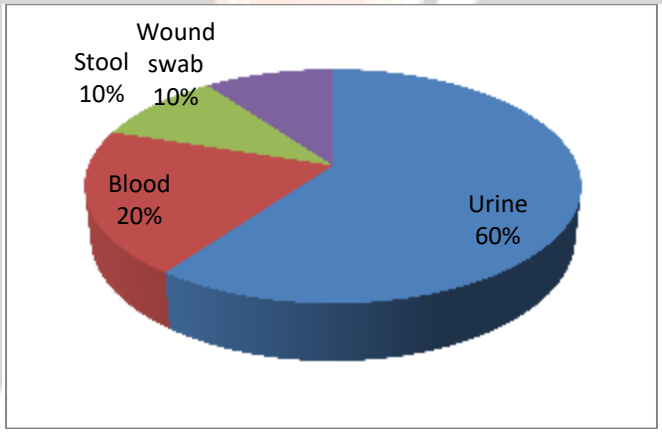

Fig 1: Pie chart showing the sample wise distribution of clinical isolates of Enterococcus spp.

Bacterial isolates:

Out of 20 Enterococci isolates, 14/20 (70%) were found to be E.faecalis, whereas 6/20 (30%) were E. faecium. Figure 2 denotes the species wise distribution of Enterococci from clinical samples.

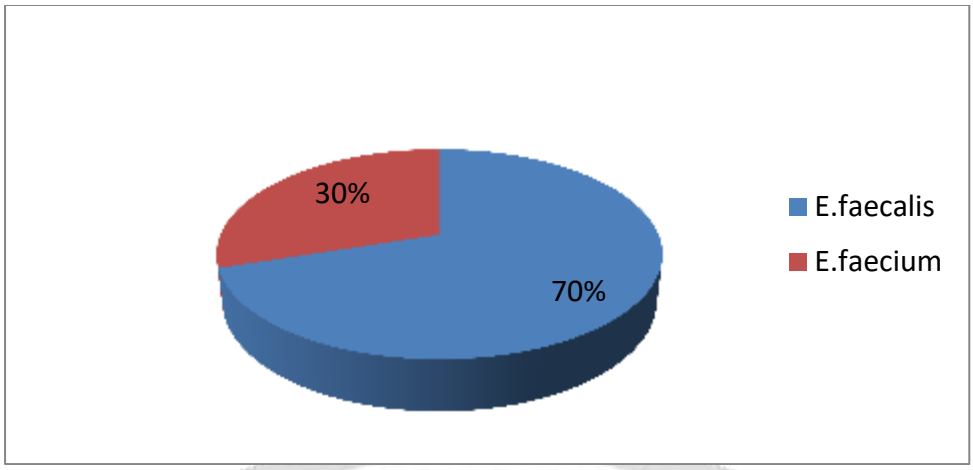

Fig 2: Pie chart showing species distribution of Enterococcus

Antibiotic susceptibility testing:

We found increased percentage of isolates were shown to be resistant to all the antibiotics used in this study. For ampicillin, amikacin, erythromycin, gentamicin, our isolates were found to resistant between 80-90%. Better sensitivity was observed in linezolid, teicoplanin and vancomycin antibiotics. The detailed results of antibiotic sensitivity patter of Enterococci was given in table 1.

Antibiotics	Sensitivity	Intermediate	Resistance
Ampicillin	1(5%)	2(10%)	17(85%)
Vancomycin	15(75%)	1(5%)	4(20%)
Teicoplanin	12(60%)	3(15%)	5(25%)
Erythromycin	2(10%)	0	18(90%)
Ciprofloxacin	6(30%)	0	14(70%)
Amikacin	1(5%)	1(5%)	18(90%)
Gentamycin	2(10%)	2(10%)	16(80%)
Tetracycline	4(20%)	4(20%)	12(60%)
Linezolid	18(90%)	1(5%)	1(5%)

Table 1: Results of antibiotic sensitivity patter of Enterococci

Results for β-lactamse by tube method:

In this method, we have observed 4/20 (20%) of our Enterococcus isolates were found to be β-lactamse positive.

Discussion:

In the recent scenario, enterococus has become more common reason for increased mortality and mordibity in the hospitalised patients. There are different infections caused by enterococcus such as urinary tract infection, endocarditis,wound infection, soft tissue infection and bacteremias. The main concern in the enterococcus is high drug resistance that has been reported. Resistance to beta lactamase and vancomycin resistance has resulted in mortality rates of 73%.

However according to our study, vancomycin and linezolid still remains the drug of choice although it appears that resistance is slowly appearing. Study conducted by Shyamala Devi in 2002 from Manipal reported 20% of

isolates were resistant to penicillin groups. In concordant to her study, we also reported the same percentage of resistance. [11]

CONCLUSION:

As enteroccocus species are frequently isolated from different clinical specimen and the rate of drug sensitivity to different antibiotics have been reduced a lot due to various mechanisms. It is very important role in the selection of drugs and better treatement outcomes.

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