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MULTIVARIATE ANALYSIS OF ANTIBIOTIC RESISTANCE PATTERN AMONG PSEUDOMONAS ISOLATES CAUSING CSOM

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ABSTRACT Chronic suppurative otitis media is one of the most common bacterial infection seen in the field of otolaryngology and the occurrence of otitis media seems to have been rising probably because of prevalence of multidrug-resistant Pseudomonas aeruginosa in the pathogenesis of otitis media(Jae-Jun Song, MD; Byung Don Lee et al). Infections caused by pseudomonas aeruginosa are often severe and life threatening and are difficult to treat because of the limited susceptibility to anti microbial agents. (Carmeli; Y; et al ; 1999). The present study was undertaken to detect the group I inducible β - Lactamase production, extended spectrum of β -Lactamases (ESBLs) production and evaluate the synergic effects antibiotic combination among the MDR pseudomonas aeruginosa isolates.
Out of 43 pseudomonas isolates 23 were multi drug resistant strains. Production of inducible p lactamase was detected (Gencer, etal., 2002; Miles, 1996) for 20strains; which were susceptible to cephalosporins. By using double disc diffusion test, extended spectrum β-lactamases were detected in 23 Strains. The synerigic effect between Cephalosporins and aminoglycoside &Cephalosporins and flouroquinolones were detected. It was observed that synergy between aminoglycoside and cephalosporins was found to be maximum 53.5% Plasmid profile analysis was done for all ESBL positive strains and plasmids were detected in all ESBL positive strains.

KEYWORDS :

INTRODUCTION

Pseudomonas aeruginosa is the most prevalent gram negative organism causing CSOM. In recent years Pseudomonas resistant to multi drugs including β-lactum antibiotics and extended spectrum of Cephalosporin is of great concern to ENT surgeons. Pseudomonas aeruginosa resists β lactam antibiotics by synthesising β lactamases. The Subsequent generation of cephalosporins which could overcome β lactamases are called extended spectrum of cephalosporins which include oxyimino β lactum like Ceftazidime and cefotaxime. Resistance to these antibiotics are by synthesis of extended spectrum of β lactamases (ESBL) which are plasmid mediated. These enzymes different from their parent enzymes by only few amino acids portion but can hydrolyse extended section of cephalosporins.

Production of various enzymes by them may require alteration in the management profile. Hence we believe that our study will definitely enlighten the management of multidrug resistant pseudomonas infection in middle ear by which we can reduce the hearing loss in many patientsof developing countries like India.

MATERIALS AND METHODS

A total of 43 pseudomonas aeruginosa isolates from chronic suppurative otitis media patients were isolated and identified by standard microbiological methods. Antibiotic susceptibility testing was done by Kirby - Bauer disc diffusion method.

DETECTION OF GROUP I INDUCIBLE BETA-LACT AMASES

Inducible Beta Lactamase was investigated by disc approximation method (Miles, 1996; Qin, et al, 2004; Collee, 1996). Cefotaxime (30 ug) disc was placed at distances 25 and 20 mm respectively from a central cephoxitin (30 pg) disc on Muller-Hinton agar plate inoculated with the test organism. After overnight incubation, distinct flattening of the inhibitory zone around the ceftazidime disc on the side nearest to the cephoxitin disc was regarded as the presence of inducible β lactamase.

DETECTION OF EXTENDED SPECTRUM BETA LAC TAMASE (ESBL)

Strains were screened for presence of ESBLs by the double-disc synergy method (Miles, 1996). Three cefotaxime (30 ug) discs were placed at distances 20, 15, and 10 mm, respectively, from a central amoxicillin-clavulanic acid disc. The test result was considered positive when an enhancement of the inhibition, zone around at least one of the cefotaxime disc toward the clavulanic-acid disc was observed as described by Bert (Bert et al, 2003).

INVESTIGATION OF SYNERGY EFFECTS OF ANTIBIOTIC COMBINATIONS

The synergy effects of the antibiotic combinations against the selected isolates were examined by disc diffusion test (Miles, 1996; Mayer and Nagy, 1999) Two discs, each containing one or other of the two tested antibiotics, were placed at a distance of about 20 mm from each other on top of a P. aeruginosa isolate-covered agar plate. Synergy was considered to occur when there was a well-observed change (>2 mm) in the zone of inhibition. The synergy was classified as weak when a change <2 mm was observed in the zone of inhibition (Mayer and Nagy, 1999),

PLASMID DNA ISOLATION

Resistant plasmid isolation was done in all ESBL positive strains (23) by alkaline lysis method developed by Birnboim and Doly (Nucleic Acids Research 7:1513, 1979).

RESULTS

Out of 43Pseudomonas aeruginosa isolates 23 were found to be multi drug resistant (ie resistant to more than one antibiotic) (53.48%),

Production of inducible β lactamase was detected in 20 (43.5%) isolates which were sensitive to cephalosporins. Out of these 20 strains 8 (40%)were found to produce β actamases when induced with a distance of 25mm between two discs (Cefoxitin and Cefotaxime). By reducing the disc distance by 5mm all strains were induced to produce β- lactamases .

By using double disc diffusion test, extended spectrum β-lactamases were detected in 23 Strains. When the two discs (Clavulanic acid and cefotaxime) were kept 25mm apart ESBLs was detected in one (4.3%) isolate. When the distance was reduced to 15mm ,8 (34.7%) strains were found to produce ESBLs. At 10mm disc approximation, 18 (78.2%) Strains produced ESBLs. By this method we found out that 1 (4.35) strain produced only one type of ESBLs and 18 (78.2%) strains produced more than one type of ESBLs and 4 (17.3%) strains did not produce ESBLs. The mechanism of resistance may be other than production of ESBLs in these strains.

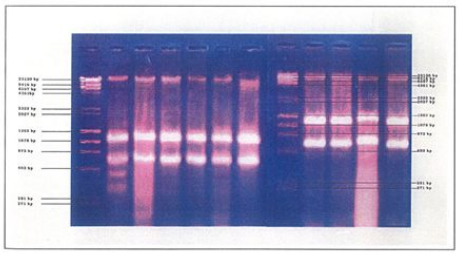
]The evaluation of the frequency of the synergic effects of the antibiotic combination was tested by the disc diffusion method. Amikacin and oflaxacin when combined with cefotaxime exhibited an expressed synergic effect in 15 (34.9%) and 14 (32.5%) isolates, weak synergic change was observed in 6 (13.9%) and 7 (16.3%)) isolates. No synergy observed in in 22 (51.2%) and 22 (51.2%) isolates.The synergy effect of ceftazidime with aminoglycoside &flouroquinolones was seen against 53.5% &50.1% of strain respectivelys.

1. Synergy effects of combinations of Aminoglycosides and fluroquinolones with third generation cephalosporins

Combination	No(%) of effective synergy	No(%) of weak synergy	No(%) of no synergy
Amikacin+Cefotaxime	15 (34.9)	6 (13.9)	22 (51.2)
Ofloxacin+ Cefotaxime	14 (32.5)	7 (16.3)	22 (51.2)
Amikacin + Ceftazidime	16 (37.2)	7 (16.3)	20 (46.5)
Ofloxacin + Ceftazidime	16 (37.2)	6 (13.9) 21(48.8)	

Resistant plasmid isolation was done in all ESBLs positive strains by alkaline lysis method developed by Birn boim andDoly. It was observed that plasmids were present in all ESBL positive strains (23).

FIGURE1. PLAMID PROFILE OF ESBL STRAINS



DISCUSSION

Pseudomonas aeruginosa is the commonest organism isolated from chronic suppurative otitis media (Berry S etal: 1996, Samiullah et al: 2005). About 43 (37.7%) pseudomonas aeruginosa were isolated in our study.

Inducibility of beta lactamases among strains sensitive to cephalosorin was detected using double disc diffusion method as described by Miles(Miles , 1996) The induction of beta lactamases was seen in 40% of isolates by using an approximation of 25 mm disc distances (Qin, et al; 2004; Miles ,1996).This rate is low when compared to the earlier observation made by Mortiz (Mortiz and Carson., 1996) who had demonstrated 68% of induction for cefotaxime using disc approximation method .But we could induce for all 100% strains after decreasing the distance between cefoxitin and cefotaxime by 5 mm.

This inducibility is significant because different beta lactamases are induced at different disc approximations, hence by decreasing the disc distance we may induce different type of betalactamases, there by proving the capacity of individual strains to induce these enzymes for producing drug resistance.

A similar method was followed for detecting the presence of extended spectrum betalactamases among resistant strains. By standard disc approximation method (25mm) we could detect ESBLs in one strain ,but by reducing disc distances we could show the presence of ESBLs in 78.2% of the strains. There are more than 192 types of beta lactamases are discovered (Bradford, 2001).These betalactamases were produced at different concentration gradients of antibiotics (Bert et al.,2003) . Some of them are produced even at low level of selective pressure produced by these antibiotics and some (require high antibiotic concentration for producing betalactamases.

By altering the distances we can vary the concentrations and in turn very selective pressures of antibiotics to pseudomonas aeruginosa. This may be possible reason for more strains producing ESBLs at reduced disc approximations According to Bert et al, the standard test with 30 mm distance is insufficient to identify most ESBLs produced by pseudomonas sp (Bert et al., 2003). Strains produced only TEM derived enzymes and one PER-1 were detected by use of a 30 mm distance .It was necessary to reduce the distance to 20 mm to detect strains that produce VEB -1 ,SHV29 and OXA 18 .Where as strains that produce the other OXA derived ESBLs were identified only at a distance of 10 or 15 mm .In the present study it was not possible to detect the types of ESBLs produced, as it may require molecular type methods .By further expanding the scope of this study ,it is possible to typing these ESBLs with the help of molecular methods and identifying the gene responsible for the resistance can be detected.

In this study synergy between cephalosporins and aminoglycosides and cephalosporins and fluoroquinolones was detected by disc diffusion method based on Kirby -Bauer' s antibiotic susceptibility testing as done by Mayer and Nagy (Mayer and Nagy, 1999).The synergy effect of ceftazidime with aminoglycoside was seen against 53.5% and the synergic effect of ceftazidime with flouroquinolones was seen against 50.1% of strains. These combinations may be useful in treating the patients with pseudomonas aeruginosa but cephalosporin aminoglycoside combination is found to be more nephrotoxic than drugs used as monotherapeutic agents.

When combination of cefotaxime with aminoglycoside and flouroquinolones were tested against pseudomonas aeruginosa drug synergy was observed in 48,8 % and 48.8 respectively. Though in this study synergy was detected with disc diffusion test. Measurement of time killing of the bacteria is the most means of assessing existence synergic effect between drugs (Mayer and Nagy).

Resistant plasmid isolation was done in all ESBL positive strains (23) by alkaline lysis method developed by Birnboim and Doly. It was observed that plasmids were present all ESBL positive strains, which may be indicative of resistant plasmid.

In conclusion pseudomonas aeruginosa can be agreed upon as the most dreaded Gram negative bacteria among isolates from chronic suppurative otitis media The main mode of resistance among these organisms is through beta lactamase and all the strains can be induced to produce beta lactamases . Through this study we detected ESBLs and plasmid among the multi drug resistant strains all the multidrug resistant pseudomonas aeruginosa. Further research on type's structure and genetic basis for production ESBL will definitely be an important improvement, which will surely have a major bearing on treatment.

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