ESBL Mediated Resistance In Pseudomonas Aeruginosa In CSOM Patients : An Emerging Threat To Clinical Therapeutics

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Abstract: Background: Chronic Suppurative Otitis Media (CSOM) is a major cause of hearing retardation in developing countries, therefore diagnosis and management of CSOM is extremely important. Objectives: To determine the antimicrobial resistance & ESBL (extended spectrum beta lactamase) producing Pseudomonas aeruginosa. Methods: The study was conducted after ethical approval by ethical committee in the Department of Microbiology, Dr. S. N. Medical College Jodhpur, Rajasthan. Two pus swabs were collected with sterile cotton swabs from CSOM patients attending ENT OPD. Identification of Pseudomonas aeruginosa was done by standard phenotypic microbiological procedure. ESBL detection was done by Combined disk diffusion method & E test. Observations & Results: Out of 150 cases, 137 (91.34%) were culture positive and no growth were obtained from 13 (8.66%) cases. Peak age of presentation was 11-20 years. The Antibiogram for P. aeruginosa revealed that resistance was maximum against Ceftazidime (75.5%) and minimum against Imipenem (6%). Among the 53 Pseudomonas aeruginosa isolate, 13 (24.5%) were ESBL producers while 40 (57.5%) were non ESBL producers. Conclusion: Our study underlines the unique problem of ESBL mediated resistance, which has created a therapeutic challenge for the Clinicians and Microbiologists. [Ramesh A NJIRM 2017; 8(1):8-12]

Key Words: Antimicrobial resistance, Cephalosporins, CSOM, ESBL, Pseudomonas aeruginosa

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Introduction: Chronic Suppurative Otitis Media (CSOM) is a major cause for hearing retardation in developing countries including India. Proper diagnosis and management of CSOM is extremely important. The common aerobic bacteria are Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Coagulase negative Staphylococcus, Proteus mirabilis and Klebsiella species[1]. The need for antimicrobial susceptibility testing is increasing day by day with rising emergence of multidrug resistant microorganisms especially P. aeruginosa[2]. It is known to exhibit intrinsic resistance to several antimicrobial agents[3]. In addition to this it also produces the enzymes, namely beta-lactamases, which are responsible for the antimicrobial resistance[4]. ESBLs are beta-lactamases that hydrolyze extended-spectrum Cephalosporins with an oxyimino side chain, Cephalosporins include Cefotaxime, Ceftriaxone and Ceftazidime, as well as the Monobactam. The ESBL enzymes encoded by the genes SHV2a and TEM have been found in P. aeruginosa and the Enterobacteriaceae family, which suggests that these organisms are widespread reservoir of the ESBL enzymes[5]. P. aeruginosa can acquire antibiotic resistance through horizontal and vertical transmission[6]. The rate of isolation of P. aeruginosa resistant to Ceftazidime, Cefepime and Imipenem has increased recently, making it more difficult to select appropriate antibiotics. Hence proper identification & management of CSOM is essential to avoid the treatment failure and recurrence.

Objectives: To study the Antibiogram of P. aeruginosa recovered from CSOM patients. We also detected the presence of ESBL producing P. aeruginosa, so as to help the Clinician in formulating an effective antibiotic strategy.

Methods: This prospective study was undertaken at the department of Microbiology Dr. S. N. Medical College & its associated hospital Jodhpur, Rajasthan. Two ear swabs were collected from clinically diagnosed CSOM patients attending ENT OPD. The first swab was used for Gram's staining and second swab was used for culture on blood agar, Mac Conkey's & chocolate agar. The culture plates were incubated aerobically at 37 °C for 18-24 hours, with 5-10% CO_2 for chocolate agar. The isolates were further identified biochemically as per the standard microbiological procedures[7]. P. aeruginosa was identified and confirm by colony morphology, gram's staining, motility testing, Oxidase test, pigment production on Pyocynin & florescein agar and growth at 42 °C. The Kirby Bauer disc diffusion method was used to study the antimicrobial susceptibility pattern of confirmed isolates using Ceftriaxone (30µg),

Ceftazidime (30µg), Ceftazidime-clavulanic acid (30µg/10µg), Ciprofloxacin (5µg), Amikacin (30µg), Piperacillin (100 µg), Ticarcillin (75µg), Aztronem (30µg) and Imipenem (10µg). The results were interpreted as per the CLSI guidelines[8].

ESBL detection: All the isolates of P. aeruginosa which showed resistance to Ceftazidime were evaluated for ESBL production by using the phenotypic confirmatory test namely combination disc & Epsilometer (E) test [9]. Briefly, a 0.5 McFarland's suspension of each isolate was spread on a Muller – Hinton agar (MHA) plate and Ceftazidime (30 µg) and Ceftazidime/Clavulanic acid (30µg /10µg) discs were placed on the agar plate. A distance of about 15mm was kept between the two discs (edge to edge) and the cultures were incubated at 37 ^{0}C overnight. The observation of ≥ 5mm increase in the zone diameter for the antimicrobial agent which was tested in combination with clavulanic acid, versus its zone diameter when tested alone, confirmed the presence of ESBL production by the organism. Further confirmation was done by E strip test, the E test ESBL strip (Ezy MIC EM098, India) carries two gradients; on the one end, Ceftazidime; and on the opposite end, Ceftazidime plus clavulanic acid. MIC is interpreted as the point of intersection of the inhibition ellipse with the E test strip edge. A ratio of Ceftazidime MIC to Ceftazidime-clavulanic acid MIC equal to or greater than 8 or CAZ ≥ 1 indicates the presence of ESBL.

Ethical Consideration: Ethical committee approval was received for this study from the Institutional Review Board of Ethical committee.

Statistical analysis: All collected data were analyzed using standard statistical method. The mean MIC value for Ceftazidime & Ceftazidime-clavulanic acid was determined.

Observations & results: A total of 150 clinically suspected patients of CSOM were included in the study. Of the cases studied, 58% were males and 42% females. A higher incidence of CSOM was seen in 11-20yrs age group as compared to other age groups, and it decreases with the increasing age of the patients. Out of these 150 cases, 91.34% were culture positive and 8.66% were culture negative. Among the culture positive, 41.2% were P. aeruginosa. An age and gender wise distribution of P. aeruginosa is shown in table 1. The Antibiogram for P. aeruginosa revealed that the maximum resistance was against Ceftazidime (75.5%) & minimum was against Imipenem (6%). Detail description shown on figure 1. All Ceftazidime resistant P. aeruginosa was tested for ESBL production by phenotypic confirmatory test. Among the 53 P. aeruginosa isolate 13 (24.5%) were ESBL producers while majority of isolates 40 (57.5%) were non ESBL producers. A total of 40 strains showed resistance to Ceftazidime, of which 13 (32.5%) were found to be ESBL producers. Comparison of Cephalosporin/ Clavulanate combined discs test and E – test In E test, only 13 Cephalosporin/Clavulanate combined discs test positive isolates were tested. Only 5/13 (38.5%) isolates were ESBL positive by this test. In the present study all Cephalosporin/Clavulanate combination disks test positive ESBL isolates were tested by E strip method and interpretated the MIC of all isolates. The standard range of MIC for CAZ was 0.5 to 32 and MIC for CAZ+ was 0.064 to 4µg/ml. Out of 13 Cephalosporin/Clavulanate combination disks test positive isolates, 6 were not conclusive, 5 were ESBL positive and 2 were ESBL negative by E test. Our interpretation of MIC for CAZ+ according to E test was shown in table no 2. Figure 2 showing ESBL production by E test. The mean MIC values in the E test were recorded as 0.92µg/ml and standard deviation was 0.49 for CAZ+.

Table 1: distribution of P. aeruginosa in different age groups and gender

Age (years)	Male			Female			Total		
	No of pt.	No of +ve isolate	% of p. aeruginosa	No of patient	No of +ve isolate	%	No of patient	No of +ve isolate	%
0-10	20	4	20	9	3	33.3	29	7	24.1
11-20	20	16	80	17	4	23.5	37	20	54
21-30	13	5	38.5	19	8	42.1	32	13	40.6
31-40	10	2	20	8	3	37.5	18	5	27.8
41-50	4	3	75	5	3	60	9	6	66.7
51-60	5	2	40	3	1	33.3	8	2	25
61-70	3	1	33.3	2	1	50	5	3	60

Table 2: ESBL isolates by Cephalosporin/clavulanate combined discs test and E test

Organism	ESBL isolate by combined disc test	Ratio of (CAZ/CAZ+) by E test		
		ESBL ≥8	Non ESBL<8	NC(Non conclusive)
P. aeruginosa	13	5	2	6

Figure 1: Sensitivity pattern of Pseudomonas aeruginosa isolated from CSOM cases

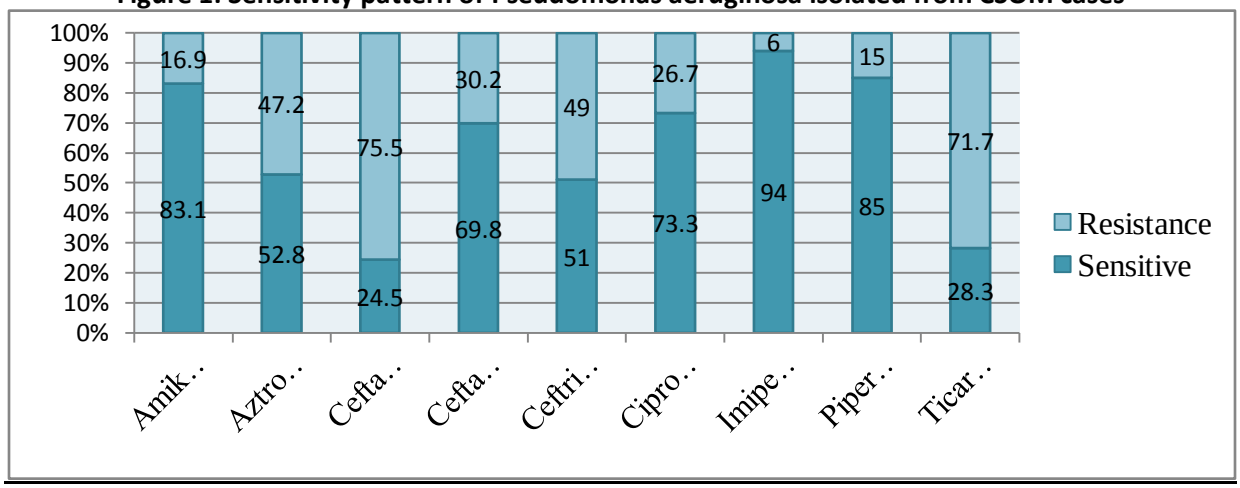

Fig 2: E test showing ESBL producing Pseudomonas aeruginosa

Discussion: The emergence of antibiotic resistant bacteria is threatening the effectiveness of many antimicrobial agents. It increases the hospital stay of patients leading to an increased economic burden on them. In the present study, Pseudomonas aeruginosa (41.1%) was the most predominant organism isolated from CSOM patients in this region and this is in accordance to study reported by Susmita et al[10] but in contrast to Lakshmi et al[11] who found P. aeruginosa was the second most common organism, next to Staphylococcus aureus in their study. In CSOM, intense secretory immunoglobulin A and immunoglobulin G (IgG) coating of bacteria is common but when Pseudomonas species is the causative agent of the infection, no bacterial coating is seen and makes them difficult to eradicate, Hanes it is frequently isolated from CSOM patients.

In the present study, the Antibiogram of 53 isolates of P. aeruginosa showed maximum resistance against Ceftazidime (75.5%), which was comparable (78.4%) to observations by Umadevi S et al[12]. Our findings differed from those of Ibukun et al, who reported a very high susceptibility (79.4%) for Ceftazidime[3]. In the present study Imipenem showed good anti Psuedomonal activity, only 6% P. aeruginosa was resistance to Imipenem. In the present study 24.5% P. aeruginosa isolates was ESBL Producers by Cephalosporin/Clavulanate combined disks diffusion test which was consistence with Peshattiwar et al[13] & Aggarwal R et al[14]. Who reported 22.2% & 20.27 %

ESBL producer in their studies respectively. Bharti et al [15] found slightly higher (32.75%) ESBL producer as compare to our study. Many studies from India have reported a very high prevalence of ESBL among P. aeruginosa like Mathur et al (64%)[16] From South India, Bakshi et al (50%)[17] from Punjab, Goel V et al (42.3%)[18] from AIIMS, New Delhi and Al-Agamy MH et al (40%)[19] from Coimbatore. Surprisingly, Jacobson et al[20] depicted a very low rate (7.7%) of ESBL production in P. aeruginosa. The prevalence of ESBL producing P. aeruginosa varies widely from place to place in India. This emphasizes need for regular monitoring of antibiotic resistance & measures to be undertaken to control their inappropriate use in any setup.

The Cephalosporin/Clavulanate combined disks test is the most widely used test due to its simplicity and ease with which the results can be interpreted. It is reliable method of ESBL detection. The comparative analysis of ESBL producing P. aeruginosa by Cephalosporin / Clavulanate combined disks and E test revealed a marked difference between the two methods. In our study 13 Cephalosporin/Clavulanate combined disks positive isolates were tested by E test 5 out of 13 (38.5%) isolates were ESBL positive by this test that was accordance with the Bharti et al[15] detected 8/30 (26.7%) ESBL positive isolate by E test. Therefore Cephalosporin/Clavulanate combined disks test was more sensitive as compared to E test.

E–Test is not suitable when the drug concentration on the strip is less than the MIC of Cephalosporin. Accordingly, higher concentration might have an inhibitory action. In our study many (5) of isolate having low MIC that probable contribute to low proportion of ESBL producers by E-test. The mean MIC values in the E test were recorded as 0.92µg/ml for CAZ+. There is wide variation in prevalence of ESBL producing P. aeruginosa in different hospital setup. It would be appropriate to study the factors that contribute to such variation this may give insight to further strategy to prevent the increasing resistance in P. aeruginosa

Conclusion: The present study underlines the problem of ESBL mediated resistance in P. aeruginosa that paces that causes a therapeutic challenge for the clinicians. To overcome the problem of emergence and spread of multidrug resistant P. aeruginosa, the microbiologists, clinicians and infection control team has to join their hands together for successful management of these patients. The ESBLs are becoming increasingly complex and diverse; we recommend the routine surveillance of antibiotic resistance in the hospital for monitoring the trends of MDR strains.

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