Nosocomial Infections with Extended Spectrum Beta Lactamase Producing Enterobacteriaceae in Pediatric Intensive Care Unit of Zagazig University Hospitals

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ABSTRACT

Enterobacteriaceae are of great concern because antimicrobial therapy of infections due to these resistant pathogens remains a clinical dilemma in hospitalized patients. It is also noted that there is an increase in the antibiotic resistance among Gram negative bacilli to third generation cephalosporins which is caused by expression of ESBL enzymes. Therefore, infections due to ESBL producing isolates continue to pose a challenge to infection management worldwide. The present study was conducted to highlight ESBL production among Eenterobacteriaceae isolated from nosocomial infections (NI) acquired in PICU of Zagazig University hospital by phenotypic and molecular method. The study was done on 604 PICU patients. Specimens for cultures were obtained according to site of NI: blood, urine, CSF, endotracheal tube (ET) aspirates and tips. isolates were confirmed by API 20E and subjected to double disc diffusion test for Phenotypic detection of the extended spectrum beta lactamases. The SHV genes were amplified by PCR, each on a 930 bp fragment. Resulting amplicons were subjected to restriction enzyme digestion for genotypic detection of SHV ESBL. From positive 96 specimens, 68 Enterobacteriaceae were isolated. The most numerous isolated enterobacteria were klebsiella spp (40.6 %), followed by E.coli (9.4%), Enterobacter Spp and Proteus spp (6.3% each), Serratia spp(5.2%), and Citrobacter spp (3.1%).66.2% of Enterobacteriaceae were ESBL producing isolates. Klebsiella pneumonia showed the highest percentage of ESBL producing strains (84.6%), followed by Citrobacter spp (66.7%), Serratia (60%), Enterobacter (50%), Porteus (33.3 %), and the least ESBL producer was E.coli (22.2%). There is high significant difference between ESBL and Non ESBL producing organisms as regarding the presence of SHV ESBL type gene. 41 Out of 45 isolates (91.1%) of phenotypically ESBL producing Enterobacteriaceae carried the SHV ESBL type gene as indicated by presence of 2 bands of 768 and 162 by RFLP. This study concludes that extended spectrum beta lactamase (ESBL) producing Enterobacteriaciaeae should be put in mind while dealing with specimens of PICU. Double disc diffusion test is a simple and sensitive confirmatory test for ESBL detection. Also, PCR-RFLP is a rapid test for genotypic ESBL detection but needs molecular equipments and facilities.

INTRODUCTION

Nosocomial infections (NIs) are infections that become clinically evident after 48 hours of hospitalization. They cause considerable morbidity and mortality, and are associated with prolonged hospital stay and increase healthcare costs.[44] The occurrence of NI differs in different patient populations and different hospitals.[5]

The background knowledge of antimicrobial susceptibility pattern of common pathogens in a given area helps to inform the choice of empirical antibiotic therapy.[45] The extended spectrum beta lactamase (ESBL) enzymes have been reported in a number of species of Gram-negative bacteria. The ESBL are capable of hydrolyzing and inactivating a wide variety of beta-lactams, including third generation cephalosporins, penicillin and aztreonam, but are susceptible to beta-lactamase inhibitors such as clavulanate, sulbactam, and tazobactam[42] Most commonly ESBLs are derived from genes of narrower spectrum TEM-1and SHV-1 lactamases by mutations that alter the amino acid configuration around the active site of these enzymes.[34]

ESBL producing organisms are reported to account for a significant proportion of intensive care infections. [36] Problems related to ESBLs have led to limited as well as expensive treatment options, and have impacted negatively on clinical outcomes.[29] In addition, NIs due to

ESBL producing organisms have been known to cause high mortality.[20]

Enterobacteriaceae are of great concern because antimicrobial therapy of infections due to these resistant pathogens remains a clinical dilemma in hospitalized patients.[22] It is also noted that there is an increase in the resistance among Gram negative bacilli to third generation cephalosporins which is caused by expression of ESBL enzymes. Therefore, infections due to ESBL producing isolates continue to pose a challenge to infection management worldwide.[32]

The present study was conducted to highlight ESBL production among *Enterobacteriaceae* isolated from NIs acquired in PICU of Zagazig university hospitals by phenotypic and molecular method.

PATIENTS & METHODS

This study was done in Microbiology and Immunology Department faculty of Medicine Zagazig University and included data collected on pediatric patients in different age groups admitted to the PICU between 1st of April 2009 and end of Marsh 2010.The Zagazig PICU is 14- bedded unit. All patients in the PICU were surveyed for NIs at different body sites if they occurred 48 h or longer after admission to the PICU with no evidence that the infection was present or incubating at time of admission. [14]

On admission, sampling from blood, urine, stool, and cerebrospinal fluid (CSF) if needed, was subjected to culture. Daily clinical and laboratory search for infection with recording the site and date of infection and sampling from blood, urine, CSF, endotracheal tube (ET) aspirates and tips (according to site of nosocomial infections) plated on blood agar, Mackconkey and chocolate agar, incubated for 24 hours at 37 C. All isolates were identified according to the standard methods.

All *Enterobaceraeciacae* isolates were confirmed by API 20E and subjected to double disc diffusion test for determining the extended spectrum beta lactamases. The SHV genes were amplified by PCR, each on a 930 bp fragment, using the forward primer 5' GCC CGG GTT ATT CTT ATT TGT CGC' 3 and the reverse primer 5' TCT TTC CGA TGC CGC CGC CAG TCA' 3). Resulting amplicons were subjected to restriction enzyme digestion for the genotypic detection of SHV ESBL. [33]

-Double disc diffusion test

A 0.5 Mc Farland- turbidity suspension of each isolate to be tested was used to inoculate Muller- Hinton agar plate by swabbing it with cotton swab. The antibiotic discs; Ceftazidime (30 mg), Cefotaxime(30 mg), Ceftriaxone (30 mg) and Aztereonam (30 mg); each one was placed at a distance of 25 mm center to center away from amoxacillin /clavulanate disc (20, 10 mg respectively). The plates are incubated for 16- 18 h in 37˚ C. [21] A clear extension of the edge of the inhibition zone of any of the antibiotics towards the disk containing clavulanic acid by at least 4mm was interpreted as positive for ESBL production. If there was no extension of zones, then the test was repeated by reducing the distance to 20 mm (center to center) from the disk containing amoxicillin / clavulanic acid. If there is no extension of the edge of the inhibition zone of any of the antibiotics the organism will be considered as ESBLs non- producer. [21]

-PCR-restriction fragment length polymorphism (PCR-RFLP):

DNA Extraction:

DNA was extracted from bacterial cells using a QIAmp DNA Mini Kit with a bacterial DNA extraction protocol (QIAGEN Inc., Valencia, Calif.). The resulting DNA extracts were stored at -20°C until PCR assessment.

PCR Amplification of SHV genes:

Pure Taq Ready-To-Go PCR beads (Amersham Biosciences) are used for PCR amplification. PCR was performed with a total reaction mixture volume of 25 µl. The reaction mixture contain 3 µl aliquot of the DNA extract (50-100 ng), 80 pmol of each primer; a 200 mM each deoxynucleoside triphosphate, and 10 mM Tris-HCl (pH 8.3), 50 mM KCI, 1.5 mM MgCl2, and 2.5 U of *Taq* DNA polymerase. The gene segment was amplified using DNA thermal cycler (Biometra,Germany). Thermocycler conditions consisted of an initial denaturation of 94°C for 2 min, followed by 30 cycles each of initial denaturation at 95°C for 30 sec, annealing at 58°C for 60 sec, and elongation at 72°C for 60 sec. An additional incubation at 72°C for 7 min was added to complete the elongation (Perilli et al., 2002). [37] Negative controls with no template were included. Amplified products were stored overnight at 2-8°C Ċ or -20°C for longer storage to be used for RFLP.

Restriction Fragment Length Polymorphism analysis (RFLP):

PCR products were subjected to restriction enzyme digestion with Asu NH1 in a total volume of 20 ul mixture as follow, 10ul of the amplified product, 2ul of the l0x SE Buffer, 0.2u1 of bovine serum albumin (BSA), 1 ul of the Asu NH1enzyme and complete the reaction with sterile water to total final volume of 20ul which was incubated for 3h at 37 C. [17]

This mixture was analyzed on 2% agarose gel containing 1% ethidium bromide with DNA Molecular Weight Marker ((Bioron,Germany).) (Fig.1). Presence of restriction site in SHV specific PCR product produced 2 bands of 768 and 162 bp indicating ESBL SHV gene and its absence with no cleavage gave a full length fragment of 930 bp.

Statistical analysis: Data were checked and analyzed by using SPSS version 15. Data were presented as number and percentage. Chi-squared (X^2) or Fisher exact test (correction of X^2) was used when appropriate. P value less than 0.05 was considered statistically significant. [41]

RESULTS

During the study period, 604 patients were admitted to the PICU. 96 PICU acquired NIs were identified among12.4% of admitted patients (75 / 604). 54 of 75 NI patients had only one NI and 21 of 75 had two NIs. Table 1 shows the distribution of NIs and associated pathogens among PICU patients. The distribution of NIs by site in a descending order of frequency was: bloodstream infection (BSI) (52.1%), ventilator associated pneumonia (VAP) (28.1%), urinary tract infection (UTI) (17.7%), and meningitis (2.1%) . *Klebsiella* Spp was the most frequent pathogen isolated (40.6 %), followed by *Pseudomonas aeruginosa* (17.7%). *Klebsiella* Spp was also the most frequent organism isolated from the blood (42%), lower respiratory tract (51.9%) and was the single pathogen isolated from CSF.

As shown in table (1), from 96 positive specimens, 68 *enterobacteriaceae* were isolated. The most numerous isolated enterobacteria were *klebsiella spp* (40.6 %), followed by *E.coli* (9.4%), *Enterobacter Spp* and *Proteus spp* (6.3% each), *Serratia spp*(5.2%), and *Citrobacter spp* (3.1%).

As shown in Table (2), the percentage of ESBL among *Enterobacteriaceae* by double disc diffusion test using different antibiotic discs combination including a cephalosporin and amoxicillin- clavulanate; the best combination for Phenotypic detection of ESBL was Augmentin (AMC)+ Ceftazidime (CAZ) which detected 45 (66.2 %) out of 68 isolates of *Enterobacteriaceae*.

As shown in table (3), 66.2% of *Enterobacteriaceae* were ESBL producing isolates. *Klebsiella pneumonia* showed the highest percentage of ESBL producing strains (84.6%), followed by *Citrobacter spp* (66.7%) *Serratia Spp* (60%), *Enterobacter* Spp (50%), *Porteus Spp* (33.3 %), and the least ESBL producer was *E.coli* (22.2%).

As shown in table (4), there is no significant difference between ESBL and non ESBL producing *Enterobacteriaceae* as regarding the presence of total SHV genes. All 45 ESBL-producing *Enterobacteriaceae* (100%) and 21 isolates of 23 non ESBL producing *Enterobacteriaceae* (91.3 %) yielded positive PCR result. So SHV genes were detected in 66 (45+21) isolates out of 68 *Enterobacteriaceae* (97%).

As shown in table (5), there is high significant difference between ESBL and Non ESBL producing organisms as regarding the presence of SHV ESBL type gene. 41 Out of 45 isolates (91.1%) of phenotypically ESBL producing *Enterobacteriaceae* carried the SHV ESBL type gene as indicated by presence of 2 bands of 768 and 162 bps by RFLP (Fig.1). In contrast, among 23 of non ESBL– producer, none of the isolates had the SHV ESBL type gene.

Table1: Percent distribution of organisms isolated from different sites among patients with PICU-acquired NIs.

	Total Positive Specimens	***Blood****	***ET****	***Urine***	***CSF*****
	96	***50***	***27***	***17***	***2***
S. aureus	7(7.3%)	2(4%)	3 (11.1%)	2 (11.8)	0(0%)
Coagulase-ve staph	6(6.3%)	6(12%)	0(0%)	0(0%)	0(0%)
Other G+ve	3(3.1%)	0(0%)	2 (7.4%)	1 (5.9)	0(0%)
Klebseilla	39(40.6%)	21(42%)	14(51.9%)	2(11.8%)	2(100%)
E.coli	9(9.4%)	4(8%)	0(0%)	5(29.4%)###	0(0%)
Serratia	5(5.2%)	3(6%)	2(7.4%)	0(0%)	0(0%)
Citrobacter	3(3.1%)	2(4%)	0(0%)	1(5.9%)	0(0%)
Enterobacter	6(6.3%)	4(8%)	1(3.7%)	1(5.9%)	0(0%)
Proteus	6(6.3%)	2(4%)	0(0%)	4(23.5%)	0(0%)
Acinetobacter	3(3.1%)	1(2%)	2(7.4%)	0(0%)	0(0%)
Pseudomonas	17(17.7%)	4(8%)	11 (40.7%)#	2 (11.8%)##	0(0%)
Candida	6(6.3%)	1(2%)	2(7.4%)	3 (17.6%)	0(0%)

* :Isolated from blood and or intravenous devices
** :Isolated from ET aspirate and or tips
*** :Isolated from CSF
NIs: Nosocomial infections, RT: Respiratory tract, CNS: Central nervous system.
#: mixed with *klebseilla* Spp (8 specimens) or acinetobacter (2 specimens)
##: mixed with *Proteus* Spp (2 specimens)
###: mixed with *Candida* Spp (2 specimens).

Table (2): Rate of phenotypic ESBL detection among 68 *Enterobacteriaceae* isolates by double disc diffusion test

Antibiotic combination	***ESBL***	
	No	**%**
Augmentin (AMC)+ Ceftazidime (CAZ)	45/68	66.2
Augmentin (AMC)+ + ceftriaxone (CRO)	40/68	58.8
Augmantin (AMC)+ cefotaxime (CTX)	36/68	52.9

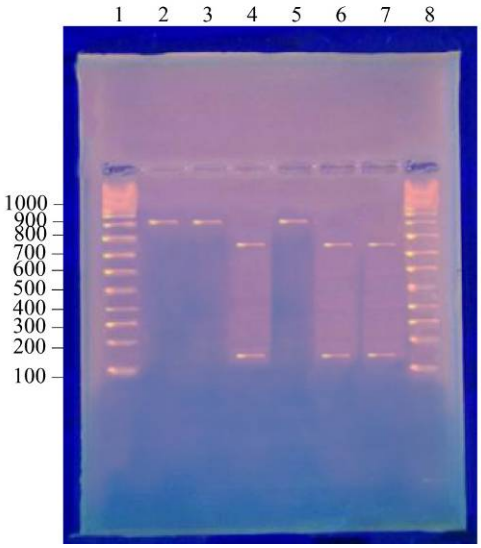

Fig (1): Ethidium bromide stained agarose gel of restriction enzyme digestion product.
Lanes 1 and 8: molecular size marker.
Lane 2, 3, and 5: 930 bp (full length fragment) means negative ESBL strain.
Lane 4, 6, 7: Two fragments 768 and 162 bp means positive ESBL strain.

Table (3): Distribution of ESBL producing and ESBL non producing *Enterobacteriaceae* by double disc diffusion test (Augmentin+CAZ).

Enterobacteriaceae	***ESBL NO 45***		***Non- ESBL NO 23***		***Total 68***
	No	**%**	**No**	**%**	
Klebsiella spp	33	84.6	6	15.4	39 (100%)
E. coli	2	22.2	7	77.8	9 (100%)
Protrus spp	2	33.3	4	66.7	6 (100%)
Enterobacter spp	3	50	3	50	6 (100%)
Serratia spp	3	60	2	40	5(100%)
Citrobacter spp	2	66.7	1	33.3	3(100%)
Total	45	66.2	23	33.8	68 (100%)

Table (4): The results of PCR amplification of SHV genes among all *Enterobacteriaceae*

PCR results	***ESBL-producer***		***Non ESBL – producer***		***Total***	***X²***	***P***	***Sig.***
	No	**%**	**No**	**%**				
Positive	45	100.0	21	91.3	66(97%)	1.56	0.21	NS
Negative	0	0.0	2	8.7	2(3%)			
Total	45	100.0	23	100.0	68(100%)			

Table (5): The percentage of SHV ESBL gene among *Enterobacteriaceae* by RFLP (PCR/Asu NHI)

PCR/Aus NHI	***ESBL-producer***		***Non ESBL – producer***		***Total***	***X²***	***P***	***Sig.***
	No	**%**	**No**	**%**				
Positive	41	91.1	0.0	0.0	41	52.78	0.001	HS
Negative	4	8.9	23	100.0	27			
Total	45	100.0	23	100.0	68			

DISCUSSION

The detection of NIs in patients admitted to PICU in pediatric hospitals indicates that such infections constitute a significant cause of illness and expense in different hospitals.[18] In this study out of 604 admitted patients, 96 (15.9 %) PICU acquired NIs were identified occurring in 12.4 % of admitted patients (75 out of 604) with some patients had one NI while others had two. In different studies for NIs in PICU reports recorded rates of 5.7%, 13.6 % , 18.3% ,23% and 45%.[18,38,3,11]

Differences among patients render the comparison of NIs among different hospitals difficult. Despite the control measures taken, higher incidence of NIs generally is found in units where in-patients have a more severe underlying disease or undergo either non-elective or high risk surgeries. [3]

In the present study, the most frequent site of NI was BSI (52.1%), followed by VAP (28.1%),, UTI (17.7%) and meningitis (2.1%). Several studies ranked BSI as the most common PICU-acquired NI representing 28, 47% and 41.3%.[38,11,16] While VAP ranked the second (28.1%) in the present study, it came in the first order with higher incidence 58.8%, 31.6 % in other studies. [18,3] This may indicate a proper implementation of rules related to tube change and ventilator use in the studied subjects .The nosocomial UTI came in the third order (17.7%) in the present study same as some studies, [26, 38] but reached 30.2% and came in the first order of NIs in another study [27]. It ranked the second NI and constitutes 28% in a study made in Alexandria University PICU [11].

The most frequent pathogens isolated in this study were Gram –ve bacilli including *Enterobacteriaceae* ((88/110, 80%), followed by Gr +ve cocci (14.5%), and yeast (5.5%). This was more or less in agreement with some studies[3,11,27] . On the contrary, other studies had shown that Gram +ve pathogens were more common than Gram –ve ones isolated from NIs[16,25,15,4] .

In this study *Klebsiella Spp* was the most frequent pathogen responsible for NIs (40.6 %) followed by *Pseudomonas aeruginosa* (17.7%).*Klebsiella Spp* was also the most frequent organism isolated from the blood (42%), LRT (51.9%). This was more or less in agreement with studies made in neonatology unit in Zagazig University[12], PICU in

Alexandria University[11], and PICU in Tiwan[27]. On contrary, in a study on ventilator associated pneumonia in Saudi Arabia, *Pseudomonas aeruginosa* was the most common isolated organism followed by *Staph aureus,*[6] and in Lithuania, *Haemophilus influenza* , *Acinetobacter Spp* and *staphylococci* were the most frequent isolates from a PICU[18].

ESBLs have emerged as an important mechanism of resistance in Gram-negative bacteria especially *Enterobacteriaceae*[40]. Delay in appropriate therapy for infections with ESBL producers not only prolongs hospital stay, but is associated with increased mortality[38]. Rapid detection of ESBL-producing organisms could therefore be advantageous, aiding appropriate antibiotic choice at the earliest opportunity and improving outcomes.

The importance of detection of ESBL production comes from the guidelines of clinical laboratory standard institute that states that isolates which have a positive ESBL phenotypic confirmatory test should be reported as resistant to all cephalosporins (except the cephamycins, cefoxitin, and cefotetan) and aztreonam, regardless of the MIC of that particular cephalosporin [35]. Unfortunately, ESBL-producing organisms often also possess resistance determinants to other important antibiotic groups, such as aminoglycosides and fluoroquinolones, leaving an extremely limited range of effective agents [28].

So we conducted the present study to highlight ESBL production among *Enterobacteriaceae*, by phenotypic and genotypic method, among nosocomially infected patients in PICU of Zagazig University Hospital.

This study showed that, the rate of ESBL detection among *Enterobacteriaceae* by double disk diffusion using the combination of augmentin and ceftazidime (66.2%) was better than using augmentin and ceftriaxone combination which detected only 58.8 % of *Enterobacteriaceae* isolates, and the poorest combination was augmentin and cefotaxime which detected only 52.9 % of *Enterobacteriaceae* isolates. The use of more than one of these agents for screening improves the sensitivity of detection.

This result agreed with many studies [45,31,1] who found that augmentin and ceftazidime combination detected more ESBL producers than the augmentin and cefotaxime combination. On contrary, Dashti et al, 2006 [10] reported a reverse result.

The present work showed that ESBL producing strains accounted for (66.2%) among all *Enterobacteriaceae*, by double disc diffusion test and this more or less agreed with other studies done in Kuwait Hospitals (75%)[10] and England Hospitals (58%) [19] but disagreed with two studies done in Zagazig University Hospitals [1,13] who reported lower incidence (32 and16%, respectively). This difference in incidence may be due differences in specimen source**.** Our study is done in PICU where antibiotic resistance is a major problem.

In this study, among the isolated *Enterobacteriaceae* (68 isolates), *K pneumoniae* was the most ESBL producer (84.6 %). A study done in neonatal intensive care unit of Mansoura University Hospitals reported that 66.7% of the isolated *Klebsiella Spp* was ESBL producer [2]. Also another study found 73.7% of the isolated *Klebsiella Spp* from Suez Canal University Hospital was ESBL producer [30]. However, these results disagreed with a study found that most ESBL *Enterobacteriaceae* isolates were E.coli. [43].

Most of ESBL are derived from TEM-1 and SHV-1 by mutation and have spread widely among *Enterobacteriaceae* being a major cause of NI which is associated with high mortality rates especially in serious infections[37].

This study showed non- significant difference between all *Enterobacteriaceae*, whether it is ESBL (100%) or non ESBL (91.3%) producers, as regard to the presence of all SHV genes. Abdel-Hady et al. (2008)[1] also found 100% of *Kebsiella Spp* had SHV-1 and SHV-2 genes. Thus SHV genes are universal in this genus[7]. The SHV-1 gene is found on chromosome of most strains of *K.pnemoniae* and is responsible for up to 20 % of ampicillin resistance in this species. It occurs also on transmissible plasmid so it can be transferred easily and spread to different members of *Enterobacteriaceae*. Mutation in SHV-1 resulted in emergence of extended spectrum beta lactamases. This occurred by one or more amino acid substitution around the active site of the enzyme, most commonly at positions 238 or 238 and 240giving to more than 60 types of SHV-ESBL[24].

Many different molecular tests have been proposed for the detection of SHV-ESBL enzymes. In this study PCR –RFLP was used and proved to be simple and rapid technique. This method allows detection of single mutation

Gly238 Ser by use of Asu NH I enzyme which is known to distinguish the majority of SHV-ESBL from SHV-1 [33].

This study showed significant difference between ESBL and non- ESBL producing organisms as regard to the presence of SHV ESBL type. Out of 45 isolates of *Enterobacteriaceae* that were ESBL-producer by the phenotypic method, 41 isolates (91.1%) carried the SHV ESBL type gene as indicated by RFLP. In contrast, among 23 isolates of non ESBL– producer, none of the isolates had the SHV ESBL type gene.

This result was more or less in agreement with a previous study who found 100% of all ESBL *K. pneumoniae* yielded positive PCR – RFLP[2]. Also, other two studies reported 80% and 95% respectively of their isolates yielded positive PCR –RFLP [1,8]. In this study, 4 isolates were phenotypically positive but yielded no SHV ESBL type by restriction enzyme. This ESBL phenotype is probably of another types of ESBLs. Others reported ESBL types include TEM, CTX-M and OXA. Of note, the prevalent genotypes vary in different countries on the basis of the differences in the use of antimicrobial agents and prevalence of plasmids that harbor ESBL genes [23,39].

This study concludes that extended spectrum beta lactamase (ESBL) producing *Enterobacteriaciaeae* should be put in mind while dealing with specimens of PICU. Double disc diffusion test is a simple and sensitive confirmatory test for ESBL detection. Also, PCR-RFLP is a rapid test for genotypic ESBL detection but needs molecular equipments and facilities.

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العدوي المكتسبة بالمستشفيات في وحدة العناية المركزة للأطفال بمستشفيات جامعة الزقازيق معزولات الإنتيروبكتريا المنتجة لإنزيمات البيتالكتاميز واسعة المدى

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تنبع أهمية مجموعة الإنتيروبكتريا من أن علاجها يمثل مشكلة في المرضي المنومين بالمستشفيات. و من الملاحظ أن هناك زيادة فى مقاومتها للمجموعة الثالثة للكيفالوسبورين لإنتاجها انزيمات البيتالكتاميز واسعة المدى. يهدف البحث الي دراسة معزولات الإنتيروبكتريا المنتجة لإنزيمات البيتالكتاميز واسعة المدى المعزولة من عينات العدوي المكتسبة من المستشفيات المأخوذة من مرضي العناية المركزة للأطفال بالمستشفي الجامعي بالزقازيق بالطرق الظاهرية و الجينية.

أجريت لدراسة علي ٦٠٤ مريض منوم بلعناية المركزة للأطفال. تم أخذ العينات طبقاً لموقع العدوي مثل الدم، البول، السائل النخاعي، إنبوب التنفس. تم التأكد من معزولات الإنتيروبكتريا بطريقة أي بي آي ٢٠ إي وأخضعت لإختبار القرص المزدوج للكشفِ الظاهرية عن إنتاجها انزيمات البيتالكتاميز واسعة المدى. و وأخضعت ايضا لعمل التفاعل المتسلسل لإنزيم البلمرة لتكبير الجينات إس إتش في. ثم أُخضع ناتج التكبير إلىهضمِ بإنزيمِ التقييدِ للكشفِ الجيني عن إنتاجها انزيمات البيتالكتاميز واسعة المدى.

و كانت النتائج كالأتى: تم عزل ٦٨ معزولة مِن مجموعة الإنتيروبكتريا من ٩٦ عينة. كانت (٤٠,٦ %) من معزولات الإنتيروبكتريا كليبسيلا،(٩,٤ %) اي كولاي، ،(٦,٣ %) لكل من الإنتيروباكتر و البروتيس. ،(٥,٢ %) سريتيا و ،(٣,١ %) ستروباكتر. كان ٦٦,٢ % من معزولات الإنتيروبكتريا منتجة لإنزيمات البيتالكتاميز واسعة المدى. و كان ميكروب الكلبسيلا (٨٤,٦ %)الأكثر انتاجا لإنزيمات البيتالكتاميز واسعة المدى ، ثم الستروباكتر(٦٦,٧ %)، ثم سريتيا (٦٠ %)، الإنتيروباكتر(٥٠ %)، البروتيس (٣٣,٣ %)، و أخيرا اي كولاي(٢٢,٢%).

كان هناك فرق مهم احصائيا بين معزولات الإنتيروبكتريا المنتجة والغير المنتجة لإنزيمات البيتالكتاميز واسعة المدى و ذلك باستخدام طريقة التعدد الجيني لإنزيم البلمرة حيث أن ٩١% من المعزولات المنتجة للإنزيمات البيتالكتاميز واسعة المدى ظاهريا كانت ايجابية.

خلص البحث الى ان معزولات الإنتيروبكتريا المنتجة لإنزيمات البيتالكتاميز واسعة المدى يجب أن توضع في الأعتبار عند فحص العينات المأخوذة من مرضي العناية المركزة للاطفال. أن إختبار القرص المزدوج للكشفِ الظاهري عن انزيمات البيتالكتاميز واسعة المدى سهل و حساس كما ان طريقة التعدد الجيني لإنزيم البلمرة للكشف الجينى عن إنتاج انزيمات البيتالكتاميز واسعة المدى طريقة سريعة و لكن تحتاج تجهيزات المعامل الجزيئية.