Phenotypic and Genotypic Detection of Extended Spectrum Beta Lactamase Klebsiella Pneumoniae Isolated from Intensive Care Units in Assiut University Hospital

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ABSTRACT

Extended spectrum β lactamases producing Klebsiella pneumoniae (ESBL-KP) are an important cause of nosocomial infections in Intensive Care Units (ICUs). We conducted a prospective study on 650 patients who were admitted to different adult ICUs at Assiut University Hospital to determine the incidence of ESBL-KP by phenotypic and genotypic methods. Phenotypic tests for ESBL were combined disc method, double disc synergy test (DDST) and E-test. Genotypic detection of ESBL bla TEM and bla SHV genes was carried out by polymerase chain reaction amplification (PCR). The overall nosocomial infection incidence rate was 20% (130 patients). Klebsiella pneumonia was isolated from 44 patients (34%), in which 23 isolates were found to be phenotypically ESBL producers. ESBL-KP was most frequently isolated from chest ICU (47.8%) and blood was the most frequent site of infection (8 isolates, 34%). Based on Clinical and Laboratory Standards Institute (CLSI) screening test for ESBL, the combined disc method was the most sensitive (23/23, 100%) followed by the E-test (95.6%) and lastly the DDST (91.3 %). SHV gene was present in 8 isolates, TEM gene in 2 isolates, both SHV and TEM in 11 isolates and none of TEM or SHV in 2 isolates. Out of 950 environmental samples, Klebsiella pneumoniae was isolated from 48 samples (16.4%) in which 7 isolates (14.5 %) were ESBL and genotyping revealed SHV in 4 strains, and both SHV and TEM in 3 strains. Conclusion: This study revealed the high incidence of ESBL-KP in adult ICUs. SHV genotype was more prevalent than TEM type. Strict implementation of basic infection control measures seems to be the most effective means for controlling the spread of ESBL organisms.

INTRODUCTION

Klebsiella pneumoniae (K. pneumoniae) is an important opportunistic pathogen which frequently causes infection in immunocompromised and debilitated patients. It is commonly associated with a wide range of hospital acquired infections including bacteremia, respiratory tract and urinary infections(3).

K. pneumoniae is naturally susceptible to third generation cephalosporines and monobactams. These organisms produce extended spectrum β lactamases (ESBLs) that hydrolyze the amide bond in the β lactam antibiotics and cause resistance to the extended spectrum cephalosporins (e.g. cefazidime, cefotaxime and ceftriaxone) and monobactams (e.g. aztreonam)(4). Most of these ESBLs are derivatives of the narrow spectrum TEM and SHV type β lactamas, with one or more amino acid substitutions surrounding their active site, thus explaining their hydrolysis profile. Now the evolution of ESBLs has resulted in more than 200 different enzymes classified in several groups. The most abundant types are represented by SHV, TEM, CTX-M and OXA(4).

Infection with ESBL producing bacterial strains are encountered singly or in outbreaks, especially in critical care units(5). Systemic infections with ESBL producing Enterobacteriaceae are associated with severe adverse clinical outcomes(6-8). A serious challenge facing clinical laboratories is that clinically relevant ESBL mediated resistance is not always detectable in routine susceptibility tests. Failure to detect these enzymes has contributed to their uncontrolled spread and some times therapeutic failure(9). It is thus essential to have updated methods for the detection of ESBL-producing strains and more specifically to detect the local epidemiology of ESBL genotype(10).

Few studies were done on the prevalence of ESBL-KP and their genotype in Egypt. Two recent studies demonstrated 66.7% & 58% of Klebsiella pneumoniae strains isolated from
neonatal intensive care units in Mansoura & Cairo University hospitals respectively were ESBL producers (11, 12). This study was carried out to:(i)-detect the incidence of ESBL-KP in ICUs in Assiut University Hospital,(ii)test the frequency of TEM and SHV genes acquisition among ESBL-KP isolates, (iii) examine the ability of different phenotypic methods to detect ESBL-producing strains in relation to CLSI ESBL screening test.

SUBJECTS & METHODS

Subjects:
This study included 650 patients admitted to eight adult intensive care units in Assiut University Hospitals, which were Coronary Care Unit, Internal Medicine ICU, Chest ICU, Tropical ICU, Neurology ICU, Neurosurgical ICU, Trauma ICU and General ICU from the period from February, 2007 to August, 2008.

Bacteriological samples were collected from 130 patients who suffered from different nosocomial infections (infection acquired 72 hours after admission). The study was revised and approved by the institutional ethics committee.

Environmental Survey:
A total number of 950 environmental samples were collected simultaneously from the surgical instruments, surfaces, walls, furniture, beds, shelves, resuscitation equipment, and trolleys of the ICUs of Assiut University Hospitals.

Collection and transportation of the specimens:
All samples were collected under complete aseptic conditions and were processed in the Microbiology laboratory of the Infection Control Unit, faculty of Medicine, Assiut University. All Klebsiella isolates were identified according to the conventional microbiological methods (Gram stain, motility, production of indole, urease test, growth on citrate as a single carbon source, Triple sugar iron, methyl red test and the voges proskauer test) up to the species level (13). Klebsiella isolates were stored in glycerol cultures.

Antimicrobial susceptibility test:
Antimicrobial susceptibility was determined by Kirby-Bauer's disc diffusion method on Mueller-Hinton agar according to the specification of Clinical and Laboratory Standards Institute (formerly the National Committee for Clinical Laboratory Standards (NCCLS)) (14). The used antibiotic disks were ampicillin, amikacin, piperacillin, ciprofloxacin, amoxicillin/clavulanic acid, cefotaxime, ceftazidime, cefepirazone, cefazoline, cefpodoxime, ceftriaxone, cefuroxime, aztreonam, gentamycin and imipenem. (Oxoid, Basingstoke, United Kingdom).

Screening for ESBL:
This was done according to the criteria recommended by the CLSI. Isolates with reduced susceptibility to one or more of the oxyimino-cephalosporines; ceftazidime (30µg) ≤ 22 mm, cefotaxime (30µg) ≤ 22 mm, ceftriaxone (30µg) ≤ 25 mm, cefpodoxime (30µg) ≤ 25 mm or aztreonam (30µg) ≤ 27 mm (Oxoid) were considered to be potential producers of ESBL (15).

Phenotypic detection of ESBL production:
1- Combined Disk method:
Production of the ESBL was detected using disc diffusion method to cefoperazone alone versus cefoperazone/sulbactum (Oxoid). ESBL production is defined as an increase of ≥ 5 mm in a zone diameter around the cefoperazone/sulbactum disks compared to zones of corresponding disks without sulbactam (16).

2- The double disc synergy test (DDST):
DDST was employed by placing cefotaxime (30µg), cefotazidime (30µg), cefepime (30µg), and aztreonam (30µg) disks (Oxoid) were placed around an augmentin disk (amoxicillin 20 µg plus 10 µg clavulanic acid) at a distance of 30 mm center to center on a lawn culture of K. pneumoniae isolate on Muller Hinton agar plate. A clearly visible extension of the edge of the inhibition zone of any disk towards the amoxicillin-clavulanic acid disk was interpreted as phenotypic evidence of ESBL production (17).

3- E-test ESBL:
The production of ESBL was also detected by using E-test ESBL strips (PDM Epsilmeter, AB Biodisk, Solna, Sweden) as described by Nuesh-Inderbinen et al. (18). Briefly the procedure for E-test ESBL involved application of one strip containing cefotazidime (TZ) gradient at one end (0.5-32µg/ml), and a gradient of cefotazidime (0.064-4 µg/ml) plus clavulanic acid (4µg/ml) at the other end (TZL). The strain was considered ESBL producer if MIC ratio of TZ/TZL was ≥ 8. The strains were considered as ESBL non producers if the ratio of TZ/TZL was < 8.
Molecular detection and identification of ESBL bla<sub>TEM</sub> and bla<sub>SHV</sub> genes by Polymerase chain reaction (PCR) amplification.

PCR amplification of ESBL genes bla<sub>TEM</sub> and bla<sub>SHV</sub> was carried out according to the method described earlier<sup>18,19</sup>. Genomic DNA was extracted from ESBL K. pneumoniae isolates from an overnight broth culture using a DNA purification kit (QIAGEN, Hilden, Germany).

Oligonucleotides primers (Clinilab, USA) used for PCR assay were as follow where primers A and B were specific for bla<sub>TEM</sub> and primers C and D were specific for bla<sub>SHV</sub>.

Primer A:
5-ATA AAA TTC TTG AAG ACG AAA-3
Primer B:
5- GAC AGT TAC CAA TGC TTA ATC A-3
Primer C:
5- GGG TAA TTC TTA TTT GTC GC-3
Primer D: 5- TTA GCG TTG CCA GTG CTC-3

For a total of 25 µl reaction volume, the following material were added in a thin walled PCR tube on ice: 12.5 µl of Taq PCR master mix (Promega, USA) after being briefly vortexed. The primer solutions were thawed on ice and mixed well before use. One µl of each primer was added to the PCR tube. One µl of template DNA was added to each tube. 9.5 µl of water, nuclease-free were added. The samples were gently vortexed and briefly centrifuged to collect all drops to the bottom of the tube. The samples were overlaid with mineral oil and placed in the thermal cycler. Control negative was included in all PCR runs that included all the components of the reaction mixture with the exception of the template DNA (which was substituted with sterile distilled water). DNA amplification was carried out in a Gene Amp9600 thermal cycler under the following conditions: initial denaturation for 5 minutes at 94ºC, followed by 35 cycles of denaturation at 95ºC for 60s, primer annealing at 58ºC for 60s, and DNA extension at 72 ºC for 60s, and lastly a final DNA extension step at 72 ºC for 10 minutes.

The amplified DNA was separated by 2% agarose gel electrophoresis, stained by ethidium bromide, and visualized by UV transillumination (Fisher Scientific Pittsburg, USA). The 930 bp and 1079 bp bands were that of SHV and TEM genes, respectively.

Figure (1): ESBL by PCR (TEM and SHV). M: marker 100 bp, L1, L3, and L6: both TEM (1079 bp) and SHV (930 bp). L5: TEM (1079 bp). L2: negative sample. L4: negative control.
RESULTS

Analysis of Nosocomial Infection

The study included 650 patients admitted to different ICUs in Assiut University Hospital during the study period. 130 patients developed nosocomial infection during the period of hospitalization with an incidence rate of (20%). *Klebsiella pneumoniae* was isolated from 44 cases representing 34% of all nosocomial infections in this study. 23 (52%) of the *Klebsiella pneumoniae* isolates were found to be phenotypically ESBL producers according to the criteria set by CLSI. ESBL-KP were most frequently isolated from the chest ICU (Table 1), and blood was the most frequent source of ESBL-KP isolation (8 isolates, 34%) (Table 2).

<table>
<thead>
<tr>
<th>Table 1: Distribution of nosocomial ESBL-KP in the eight ICUs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Unit</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Chest ICU</td>
</tr>
<tr>
<td>General ICU</td>
</tr>
<tr>
<td>Neurology</td>
</tr>
<tr>
<td>Neurosurgery ICU</td>
</tr>
<tr>
<td>Tropical ICU</td>
</tr>
<tr>
<td>Coronary Care Unit</td>
</tr>
<tr>
<td>Internal Medicine Unit</td>
</tr>
<tr>
<td>Trauma</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2: Distribution of nosocomial ESBL-KP among different clinical samples</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type of sample</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Blood</td>
</tr>
<tr>
<td>Endotracheal tube aspirates</td>
</tr>
<tr>
<td>Sputum</td>
</tr>
<tr>
<td>Urine</td>
</tr>
<tr>
<td>Rectal swabs</td>
</tr>
<tr>
<td>Bed sores</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

**Antibiotic susceptibility patterns of ESBL-Kp isolates:**

All of the ESBL-KP isolates were susceptible to imipenem, while 96% were resistant to ampicillin. Other resistant rates were 33.3% to amikacin, 50% to tetracycline, 58.2% to gentamycin, 61.1% to cefotaxime, 72.2% to ciprofloxacin, 77.8% to ceftazidime, 77.8% to cefaperazone, 83.3% to amoxicillin/clavulanic acid, 83.3% for piperacillin, 83.3% to cefazolin, 83.3% to ceftriaxone, 94.4% to cefuroxime and 94.4% to aztreonam.

**Analysis of Environmental samples:**

Out of the 950 environmental samples, 48 (16.4%) *Klebsiella pneumoniae* were detected, 7 (14.5%) of them were found to be ESBL producers (Table 3).
Table (3): Distribution of environmental ESBL-KP in the eight ICUs

<table>
<thead>
<tr>
<th>Unit</th>
<th>All Klebsiella isolates</th>
<th>ESBL-KP isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>Chest ICU</td>
<td>12</td>
<td>25%</td>
</tr>
<tr>
<td>General ICU</td>
<td>10</td>
<td>20.8%</td>
</tr>
<tr>
<td>Neurology</td>
<td>4</td>
<td>8.3%</td>
</tr>
<tr>
<td>Neurosurgery ICU</td>
<td>8</td>
<td>16.7%</td>
</tr>
<tr>
<td>Tropical ICU</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Coronary Care Unit</td>
<td>6</td>
<td>12.5%</td>
</tr>
<tr>
<td>Internal Medicine Unit</td>
<td>3</td>
<td>6.2%</td>
</tr>
<tr>
<td>Trauma</td>
<td>5</td>
<td>10.5%</td>
</tr>
</tbody>
</table>
| Total            | 48 | 100% | 7 | 100%

Performance of phenotypic ESBL detection tests:
Discrepancies between the results of different ESBL detection methods were observed. 23/23 of the phenotypically ESBL-KP isolates were detected as positive ESBL-producing strains by the combined disc method, while 21/23 were positive by the DDS test and 22/23 was positive by ESBL-E test. Based on the results of the CLSI screening test for ESBL, the combined disk method was found to be the most sensitive ESBL screening test (Table 4).

Table (4): Sensitivity of phenotypic ESBL detection methods in comparison with the results of CLSI screening test for ESBL

<table>
<thead>
<tr>
<th>Method</th>
<th>CLSI screening test for ESBL-KP</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>positive (n=23)</td>
<td>negative (n=21)</td>
<td>Sensitivity (%)</td>
<td>Specificity (%)</td>
<td>PPV %</td>
</tr>
<tr>
<td></td>
<td>True +ve</td>
<td>False -ve</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disk method</td>
<td>23</td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>DDS test</td>
<td>21</td>
<td>2</td>
<td>91.3</td>
<td>100</td>
<td>91.3</td>
</tr>
<tr>
<td>E.test</td>
<td>22</td>
<td>1</td>
<td>95.6</td>
<td>100</td>
<td>95.6</td>
</tr>
</tbody>
</table>

SHV and TEM genes detection by PCR: (Figure 1)
Genotyping of ESBL-KP isolates demonstrated TEM, SHV or both in 21/23 of patient isolates and in 7/7 of environmental isolates (Table 5).

Table (5): Frequency distribution of SHV and TEM genes in patient and environmental isolates

<table>
<thead>
<tr>
<th>The bla gene</th>
<th>Patient isolates (23)</th>
<th>Environmental isolates (7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>SHV alone</td>
<td>8</td>
<td>34.8%</td>
</tr>
<tr>
<td>TEM alone</td>
<td>2</td>
<td>8.7%</td>
</tr>
<tr>
<td>SHV+TEM</td>
<td>11</td>
<td>47.8%</td>
</tr>
<tr>
<td>none</td>
<td>2</td>
<td>8.7%</td>
</tr>
</tbody>
</table>
Epidemiological data:
The patients included in this study were bedridden and acquired the infection during their stay in the hospital. Both nosocomial and environmental ESBL-KP were most frequently isolated from the chest ICU followed by the general ICU. This greatly suggests the role of cross transmission between patients and environment by the hands of healthcare workers in ESBL-KP acquisition.

DISCUSSION
Extended spectrum β lactamases continue to be the leading cause of resistance to β lactam antibiotics among Gram negative bacteria. There has been an increased incidence and prevalence of extended spectrum β lactamases that show a wide spread in hospital settings worldwide (20). Recently, acquired resistance to oximino-cephalosporines and aztreonam in Gram negative bacilli has become a serious clinical problem. The first ESBL was discovered in Germany in 1985 (21).

In our study, 20% of the patients developed nosocomial infections during their stay in the ICU which is lower than that reported in previous Egyptian study (36.9%) (11). The nosocomial infection rate is variable all over the world ranging from 3-74% (22,23). This variability can be attributed to many factors including difference in patient underlying disease, the heavy use of broad spectrum antibiotics and patient care practices.

In this study, Klebsiella pneumoniae was isolated from 34% of the nosocomial infection (44 isolates), 52% (23 isolates) were ESBL producers. As reported in previous Egyptian (11) and European studies on ESBLs (20,24,25), E. coli and Klebsiella pneumoniae are the species where ESBL are most frequently identified. Recent studies from Sentry Antimicrobial Surveillance Program (SENTRY) showed that ESBL-KP rates vary from 1.1%-3.2% in USA to 21.2-24.7% in Europe, 34.4%-39.8% in Latin America and 29% in Pakistan (26,27). However, in a two previous Egyptian study ESBL-KP rates were (21%) and (29%) which is lower than that reported in this study (11,20). Increased prevalence of ESBL in our community is suggested to be due to intense prescription of 3rd generation cephalosporines in hospitals and the dissemination of these organisms by inappropriate hygienic measures.

The emergence and spread of ESBL-KP creates a dilemma for clinicians because of the multiple-drug resistance expressed by these organisms (29). The resistance pattern of ESBL-KP isolates in our study to different ESBL-antibiotics were 61.1% to cefotaxime, 77.8% to ceftazidime, 77.8% to cefepime, 83.3% to cefazolin, 83.3% to cefpodoxime, 88.9% to ceftriaxone, 94.4% to ceftroxime and 94.4% to aztreonam. Prior to 2002, the reported range of cefotaxime and ceftazidime resistance in cross sectional studies globally ranged between 26-49% to cefotaxime and 40-57% for ceftazidime (30-33). In a previous study conducted in Assiut in 2002, lower rate of ceftazidime resistance (33.3%) was recorded (34). The increase in the cefotaxime resistance rate between the 2 studies is consistent with what happened elsewhere. In USA the resistance rate to cefotaxime increased from (1.5%) in 1987 to (20%) in 1993 (35), in China the resistance rate increased from (18%) to (43%) between 1994 and 2001 and in a recent study conducted in India the cefotaxime resistance rate increased from (57.4%) in 2002 till it reached 100% in 2004 (36). This increase in the cefotaxime resistance rate with time may be part due to increase in the detection rate of ESBLs, but more importantly due to the intense prescription of 3rd generation cephalosporines in communities and hospitals.

None of our ESBL-KP isolates (100%) were resistant to imipenem, which agrees with results of studies from Egypt, USA, Spain, and China where 100% of their isolates were sensitive to imipenem. (37-39) However, in a striking development, a following study done in Assiut University Hospital detected 5.6% resistance to imipenem (40). The emergence of carbapenem resistance is alarming and poses a serious threat to hospitalized patients since many of ESBL producers are resistant to all other agents. On the contrary 100% of the ESBL-KP isolates in our study were resistant to amoxicillin which confirms the inefficiency of this drug in treatment of infections caused by ESBL-KP in our hospital.

In this study a high prevalence of multidrug resistant ESBL-KP isolates were observed. The resistance plasmids commonly mediate resistance to more than one antibiotic, sometimes as many as seven antibiotics (41). These findings agreed with those reported in many other parts of the world (39,42,43). However, lower rates of antibiotic resistance were detected in a previous study conducted in Assiut (34) which means the dissemination of multidrug resistant strains especially inside hospitals. The high prevalence of multidrug
resistant ESBL-KP isolated in this study combined with the overall high usage of antibiotics in Egypt may suggest that the emergence of these organisms is due to local selection of resistance strains.

The prevalence of ESBL-producing strains decreased when hospitals reduced their use of oxyimino-cephalosporines in favor of beta-lactam inhibitor plus piperacillin(44). Only 33.3% of our isolates were resistant to amikacin thus justifying its use as empiric therapy. In line with our results, a previous study conducted in Egypt reported 22.2% resistance to amikacin(11) and in USA only 11% of their isolates were resistant to amikacin(37).

The CLSI screening test was able to detect all ESBL-producing isolates in the present study. The combined disk method was able to detect 23/23 (100%) of the ESBL isolates while DDST allowed the detection of 21/23 (92%) of the ESBL isolates and the E-test detected 22/23 (96%) of the ESBL isolates. The combined disk test was the most sensitive confirmatory test in the present study and as a relatively cheap method can be used effectively in hospital settings. Similar results have been described in(45), although an Indian study(36) reported the DDST as the best confirmatory test. Discrepancies between different detection methods are probably a result of various genotypes that challenge the sensitivity of ESBLs detection methods. Only few enzymes having broad oxyimino-cephalosporines substrate profile can be easily detected by all methods(46).

More than 100 ESBL variants from different types are known. The most abundant types are SHV, TEM, CTX-M, OXA (Schmitt et al., 2007). The prevalent genotype varies in different countries with TEM an SHV enzymes are the most frequently observed(2,47). Mutations in the genes encoding these enzymes expand the spectrum of their activity to include extended spectrum cephalosporines(11). In this study, PCR could identify ESBL production genes in 21/23 of the phenotypically ESBL KP. The most commonly detected genes for ESBL-KP in this study belonged to the SHV group (19/23, 82.6%). The dominance of SHV ESBL in Klebsiella pneumoniae has been described in a previous Egyptian study where 100% of the isolated ESBL-KP had the SHV genes(11). These findings suggest that ESBL-KP SHV seems to be representative to those circulating in Egypt. In a recent study in Spain, SHV was the dominant genotype(48), while TEM genes were found to be the major genotypes of ESBL-KP in United States and in United Kingdom(2,47).

Plasmid mediated ESBLs are often reported in isolates that also produce chromosomal or plasmid-mediated non ESBL β-lactamases(59,55). However, there are few reports of simultaneous production of more than one ESBL in the same strain(48). In this study, a significant number of (11, 47.8%) of ESBL-KP isolates produced TEM and SHV simultaneously. Such coproduction has been previously reported in China(52) and in Spain(48). Other combination has also been described as CTX-M14 and SHV-12 in Korea(53), CTX-M3 and SHV-12 in a nationwide study in Canada(54) and in Spain(54). These differences between countries can be attributed to the differences in the use of antibacterial agents and prevalence of plasmids which harbor the ESBL genes.

There are many other reports from around the world of proven clonal spread or hospital outbreaks of ESBL-KP. The role of hospital environment in spread of infection is clear in this study. The highest isolation rate of ESBL-KP from patient and environmental samples is the chest ICU followed by the general ICU. In line with our findings, the studies from West Nigeria and Cameroon who suggest a serious role of hospital environment in transmission of infection(55,56).

It is essential for a diagnostic microbiology laboratory to have updated methods for the detection of ESBL producing strains(49). A failure to detect ESBLs and subsequent treatment with oxyimino-cephalosporines are associated with high risk of therapy failure(57). A little is known about the ESBL genotype and their expression profile in Egypt. More extensive studies involving genotyping of ESBL gene variants and sequence analysis will be very useful in detecting new evolving genes and the presence of new combination with the probability of clonal diffusion of such strain.

CONCLUSION

ESBL-KP represents a current problem in Assiut University Hospital. Most of theses isolates are multidrug resistant leaving few therapeutic options for the physician. ESBL-KP were also isolated from different environmental samples which means that the environment is a potential reservoir of pathogens capable of cross-transmission of such organism between patients by hands of healthcare workers with the probability a hospital outbreak. Strict
implementation of basic infection control measures seems to be the most effective means for controlling the spread of ESBL organisms.

REFERENCES


32. Cao W, Tong M H, and Wang J G (2002). Extended-spectrum beta-lactamases detection in enterobacteriaceae and


34. Mandour S M (2002). The use of bacteriocin, plasmids and serotyping as finger printing of Klebsiella. Ph.D. Thesis (Microbiology), Faculty of Medicine, Assiut University.


40. Mokhtar M A (2008): Nosocomial bacteremia in intensive care units at Assiut University Hospitals with special reference to extended-spectrum B-lactamase producing organisms. Ms Thesis (Microbiology), Faculty of Medicine, Assiut University, Egypt.


الكشف عن النمط الظاهري و الجيني لميكروب الكليسيلا المفرز لآزمي البتين لاكتاميز الواسع
المدى المعزول من وحدات العناية المركزية في مستشفى أسيوط العامي

د. أ. إ. ن. عبد المجيد هاني - د. شريف أحمد عبد الرحمن - د. سلامة أحمد سيف الدين
د. نعمة محمد كمال الشربيني - د. علاء محمد الحسني

تعتبر البكتيريا التي تفرز آزمي البتين لاكتاميز الواسع المدى من أهم المسببات لعدوى المستشفيات في العالم كله وقد
زادت نسبة هذه البكتيريا في السنوات الأخيرة. وقد انتشر آزمي البتين لاكتاميز الواسع المدى وخاصة في ميكروب الكليسيلا و
هو المسؤول عن مقاومة مجموعة الكيماوسيتربين الواسع المدى والأميبودام. وكما أن العرض من هذه الدراسة هو معرفة نسبة انتشار ميكروب الكليسيلا الرونية التي تفرز آزمي البتين لاكتاميز الواسع المدى
في عدوى المستشفيات في وحدات العناية المركزية في مستشفى أسيوط العامي وتقنيات الاختبارات الميكروبولوجية المختلفة
لإكتشاف هذا الأزمي في العمل على تحديد نوع هذا الأزمي بواسطة تفاعل البلمرة التسلسل.

وقد أجريت هذه الدراسة على 250 مريض ادخلوا إلى وحدات العناية المركزية المختلفة في مستشفى أسيوط العامي.
و أظهرت النتائج أن 130 مريضاً أصيبوا بعدوى المستشفيات و أن في هذه الحالات كان ميكروب الكليسيلا الرونية
هو المسؤول عن حدوث عدوى المستشفيات. و من بين عزلات ميكروب الكليسيلا الرونية وجد 33 ميكروب يفرز آزمي البتين
لاكتاميز الواسع المدى.

و قد تم التعرف على ميكروب الكليسيلا الرونية بواسطة المزارع و التفاعلات البويوكيميائية المختلفة. وقد تم الكشف على
قعدة ميكروب الكليسيلا الرونية على أفزاع آزمي البتين لاكتاميز الواسع المدى بواسطة اختبارات NCCLS
المؤكد، الاختبار المزدوج و كذلك اختبار LCCL.
و عند تقسيم هذه الاختبارات وجد أن حساسية اختبار NCCLS
المؤكد تقدر ب 100% بينما اختبار LCCL له حساسية تقدر ب 95.6% و الاختبار المزدوج %91.3.
بالإضافة إلى ذلك فقد تم تحديد العين المسأل على أفزاع آزمي البتين لاكتاميز الواسع المدى في ميكروب الكليسيلا الرونية
باستخدام تفاعل البلمرة التسلسل الذي أظهر أن آزمي SHV هو الأكثر انتشارا.

و قد تم أيضاً أخذ عينات من الوان و الألمات و الأثاث في وحدات العناية المركزية المختلفة في مستشفى أسيوط العامي.
و قد تم عزل 7 عزلات من ميكروب الكليسيلا الرونية التي تفرز آزمي البتين لاكتاميز الواسع المدى من العناية
المتاحة للأمراض الصدرية و الذي سجل أعلى معدلات لعزل هذا الميكروب من المرضى المصابين بعودي المستشفيات
خلال فترة الدراسة.

و نستنتج من هذه الدراسة أن عدوى المستشفيات التي يسببها ميكروب الكليسيلا الرونية التي تفرز آزمي البتين لاكتاميز
الواسع المدى تشكل مشكلة قائمة في مستشفى أسيوط العامي. و يجب اختبار NCCLS
المؤكد من الميكروب المبرّر والنقية
للكشف عن آزمي البتين لاكتاميز الواسع المدى مما يمكن إدخال جريئة روبوتيكية للكشف عن وجود هذه الأزميات في
الفحص العملي.