Evaluation of Multi-drug Resistance and ESBL, AmpC, Metallo β-Lactamase Production in Gram Negative Bacteria Causing Pharyngotonsillitis

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ABSTRACT
Tonsillitis and Pharyngitis are an infection of the throat region poses one of the most common problems in the upper respiratory tract infection and is one of the main reasons leading the persons to seek advice from a physician. This study was carried out during the period (December, 2012 to August, 2013). A total of two hundred of throat sawbs were collected from patients with tonsillitis and pharyngitis infection at the age ranged between (1-70) years, who were attending the clinic outpatient unit in Rizgary and Raparin teaching Throat swabs were collected and examined for microscopically using Gram stain examination and culture technique. Isolated microorganisms were identified using microscopical, morphological, biochemical tests and Vitek2 compact system. The results showed that 134 (67%) of throat swabs were culture positive the total number of isolated microorganism isolates obtained from patients were (185) isolates. These isolates were distributed between Gram-positive bacteria 143 (77.3%), Gram-negative bacteria 34 (18.4%) and fungi 8 (4.3%). Single isolates found in 84 (62.7%) and mixed isolates in 50 (37.3%).

All Gram-negative bacteria isolates were screened for the presence of Ampicillin resistant (AmpC) β-lactamase enzyme using Disk antagonism test. Out of (34) Gram-negative isolates 11 (32.4%) were found to be AmpC β-lactamase producers.

All Gram-negative bacteria were screened for their ability to produce Metallo β-lactamase enzyme using Imipenem-EDTA (Imipenem-Ethylene diaminetetra acetic acid) combined disc test. Out of (34) Gram-negative bacteria isolates, 23 (67.6%) were found to be Metallo-β-lactamase producers.

INTRODUCTION
A throat infection is a general term describing a disease affecting the area of land surrounding the throat. It is discomfort, pain or scratchiness in the throat due to any infectious condition. Commonly known as a sore throat, throat infections are most commonly caused by bacteria or viruses. A sore throat often makes it painful to swallow in cases of throat infection. It has been estimated that a throat
infection is one of the top ten reasons that people visit emergency rooms for treatment (Bhatia and Khutpale, 2011).

Acute pharyngotonsillitis is one of the most common infections encountered by pediatricians and family physician. Most children with acute pharyngotonsillitis have symptoms that can be attributed to infection with a respiratory virus, such as adenovirus, influenza virus, para-influenza virus, rhinovirus, and respiratory syncytial virus. However, in approximately 30% to 40% of cases, acute pharyngotonsillitis is of bacterial etiology (Brook and Dohar, 2007).

Pharyngitis and Tonsillitis are two common URTI causing ill health in children presenting to primary care physicians. Although most cases of pharyngitis and tonsillitis are viral in origin, bacterial causes are important because of the non supportive sequela like rheumatic fever and rheumatic heart disease in group a β- hemolytic Streptococcus (GABHS) infection. Rheumatic fever (RF) and its cardiac complication of rheumatic heart disease (RHD) remain major health problems in developing countries (Sadoh et al., 2008). Among various bacteria able to colonize airways, Pseudomonas aeruginosa is an opportunistic microorganism often recovered in the airways of patients with an impairment in their host defence. Among the populations having a high risk to develop Pseudomonas aeruginosa pneumonia, patients admitted to intensive care units with respiratory assistance represent the most exposed population. Another exposed population includes patients undergoing chemotherapy following cancer (Bentzmann et al., 1996).

Proteus mirabilis it was causing tonsillopharyngitis as it isolated from tonsillopharyngitis of children (Sadoh et al., 2008). Proteus mirabilis an organism that is often considered to be implicated in contamination and colonization is occasionally isolated in severe infections. In hospitals it is the second most frequently isolated Enterobacteriaceae species after Escherichia coli (Champs et al., 2000).

The ESBL enzymes are plasmid-mediated enzymes capable of hydrolyzing and inactivating a wide variety of β-lactams, including third generation Cephalosporins, Penicillins and Aztreonam. These enzymes are the result of mutations of TEM-1 and TEM-2 and SHV-I. All of these β-lactamase enzymes are commonly found in the Enterobacteriaceae family. Normally, TEM-1, TEM-2 and SHV-1 enzymes confer high level resistance to early Pencillins and low level resistance to first generation Cephalosporins. Widespread use of third generation cephalosporins and aztreonam is believed to be the major cause of the mutations in these enzymes that has led to the emergence of the ESBLs (Chaudhary and Aggarwal, 2004).

Production of ESBL has been reported in virtually all species of Enterobacteriaceae, which greatly complicates the therapy of infections caused by these organisms. However, the frequency of isolates producing AmpC β-lactamases, especially plasmid mediated AmpC (pAmpC), is largely unknown. These β-lactamases confer resistance to broad spectrum cephalosporins and aztreonam, a multidrug-resistant (MDR) profile (Silva Dias et al., 2007).

AmpC type β-lactamases (also called Cephalosporinases) are isolated commonly isolated from extended- spectrum cephalosporin-resistant Gram-negative bacteria. AmpC β-lactamase in contrast to ESBL hydrolyzes extended-spectrum Cephalosporins but is never inhibited by β-lactamase inhibitors (Barie, 2012). Resistance to Carbapenems may result from the presence of Metallo-β-lactamases (MBLs). These are zinc-dependent β-lactam-hydrolysing enzymes with broad substrate specificity that are not inhibited by clavulanic acid, sulbactam or tazobactam. MBL genes can be chromosomal- or plasmid borne, and are often located in integrons. They are usually found in non fermenting Gram-negative bacteria and are not frequent among the Enterobacteriaceae (Cendejas et al., 2010).

**MATERIALS AND METHODS**

**Sample Collection**

Throat swabs obtained from two hundred patients attending outpatient clinic unit center complaining of sore throat and fever more than 38.5°C and diagnosed as tonsillitis and pharyngitis which are collected during the period from December 2012 to April 2013 in Rizgary and Raparin teaching hospitals in Erbil city. Their age ranged between (1-70) years. Two swabs were taken from each patient by (physician). The swabs were put in transport media and sent immediately to the laboratory. One of swabs was examined directly using stain and the other was cultured immediately.
Much information was taken directly from each patient using a special questionnaire sheet. The information included:

- Patient name, age, location (residence) of stay, educational status, gender, symptoms, history of tonsillectomy, Drug history, past medical history, past surgical history

### Isolation of Microorganisms

For isolation of microorganisms, the specimen of throat swabs was directly inoculated on culture media Blood agar and MacConkey agar plates were incubated aerobically at 37°C for (24-48) hours. Chocolate agar plates were incubated microaerophilically at 37°C for (24-48) hours. Microaerophilic incubation was done using a candle jar which supplied (5-10) % CO2 (Razzak et al., 2011).

### Identification of Microorganisms

Pure colonies of isolated microorganisms were identified using morphological, biochemical tests. Species identification and antibiograms for pathogens were performed using the Vitek 2 compact system (Nagaraja, 2008).

### Antimicrobial Susceptibility Test by Vitek 2 System

The system includes an AES that analyzes minimum inhibitory concentration (MIC) patterns and detects phenotypes for most organisms tested (Kollef, 2000).

#### β-LACTAMASE DETECTION

**Detection of B- Lactamase In Gram Negative Bacteria**

A total of 73 isolates of Gram negative isolates were screened for different types of β-lactamase enzyme which were responsible for multi-resistant mechanisms in Gram negative bacteria.

**Detection of Extend Spectrum B- Lactamase (ESBL)**

**Confirmatory Test for Detection of ESBL by Double Disc Diffusion Test**

A double disc diffusion test was performed with Amoxicillin-Clavulanic acid with Ceftazidime (Umadevi et al., 2011).

- A bacterial suspension was prepared with the turbidity adjusted to a 0.5 McFarland standard.
- Mueller Hinton agar plates were streaked with the suspension according to CLSI guidelines.
- An Amoxicillin (20µg/ml)-Clavulanic acid (10µg/ml) (AMC) disc was placed at one side of the plate.
- Individual disc containing (30µg/ml) of CAZ (Ceftazidime), placed on to the plates in a distance of 1 cm edge to edge from an Amoxicillin-Clavulanic acid disc.
- The plates were incubated at 37°C for 18-24 hours.
- After incubation, an enhanced zone of inhibition between any one of the β-lactam discs and amoxicillin clamulanic acid was interpreted as a positive result.

**Table 1. The standard inhibition zone of Amoxicillin-Clavulanic acid and Ceftazidime**

<table>
<thead>
<tr>
<th>Antibiotic discs</th>
<th>Concentration µg/ml</th>
<th>Resistance mm</th>
<th>Intermediate mm</th>
<th>Susceptibility mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin-clavulanic acid (AMC)</td>
<td>20-10 (30)</td>
<td>≤ 13</td>
<td>14-17</td>
<td>≥ 18</td>
</tr>
<tr>
<td>Ceftazidime (CAZ)</td>
<td>30</td>
<td>≤ 14</td>
<td>15-17</td>
<td>≥ 18</td>
</tr>
</tbody>
</table>

#### DETECTION OF AMPC β-LACTAMASE

**Confirmatory Test for Detection of Ampc Enzymes by Disk Antagonism Test**

Tested isolates with a turbidity equivalent to that of 0.5 McFarland standards was spread over a Mueller Hinton agar plate. Ceftazidime (30µg/ml) and Cefoxitin (30µg/ml) disks were placed 20 mm apart from center to center, after overnight incubation an isolates showing blunting of the ceftazidime
zone of inhibition adjacent to the cefoxitin disk or reduced susceptibility to each of them were screened as positive for AmpC β-lactamase production (Taneja et al., 2008).

**Detection of Metallo β-Lactamase Using Imipenem (IMP) - EDTA Combined Disc Test**

The IMP- EDTA combined disk test was performed for the tested organisms were inoculated on to plates with Mueller Hinton agar as recommended by the CLSI. Two (10µg/ml) Imipenem disks were placed on the plate, and 10µl of EDTA solution were added to one of them to obtain the desired concentration (750µg/ml). The inhibition zones of the imipenem and imipenem- EDTA disks were compared after (18- 24) hours of incubation at 37˚C. In the combined disc test, if the increase in inhibition zone with the Imipenem and EDTA disc was ≥ 7 mm than the Imipenem disc alone, it was considered as Metallo β-lactamase positive (Behera et al., 2008).

Solution of a 0.5 M EDTA was prepared by dissolving 186.1 gm of EDTA. 2H2O in 1000 ml of distilled water and its pH was adjusted to 8.0 by using NaOH and sterilized by autoclaving (Yong et al., 2002).

**RESULTS AND DISCUSSION**

A total of two hundred throat swabs were collected from patients attending Rizgary and Raparin Teaching Hospitals in Erbil city suspected of having tonsillitis/pharyngitis. The results as showed in figure (1) that among 200 throat swabs only 134 (67%) were culture positive, while 66 (33%) were culture negative. The results of this study indicated that the rate of bacterial growth that detected from patients with tonsillitis/pharyngitis was 134 (67%). These results were in agreement with those dictated by Raju and Selvam, (2012) from India who found that (68%) of samples show positive cultures, Sadoh et al., (2008) from Nigeria reported of (53.42%). While in a study done in Bangladesh by Amin et al., (2009) their results showed that (26.3%) were culture positive which is lower than reported in the present study. The high rate of positive cultures of bacterial pharyngitis in our study may be due to that bacterial tonsillitis/pharyngitis may be common in Erbil city and others might due to sample size and our target populations were selected by physician only patients with symptoms of tonsillitis and pharyngitis.

**Figure 1:** Relation between culture results and tonsillitis/pharyngitis infection

The number and percentage of microorganisms isolated from 134 patients with tonsillitis/pharyngitis are shown in table (2) the most common isolated organisms was *Pseudomonas aeruginosa* 11(6.2%) and *Proteus mirabilis* 10 (5.6%). The identification of microorganisms causing tonsillitis/pharyngitis was based on cultural morphology, biochemical characteristics and Vitek2 compact system.

In the present study the antimicrobial susceptibility test done by Vitek2 compact, the results table (3) showed that among Gram-negative bacteria the most effective antibiotics that have low percentage of resistance were Tobramycin (0%), Cefepime (0%), Levofoxacin (0%) followed by Meropenem (17.6%) when used for all tested Gram-negative bacteria. While the isolates showed high percentage of resistance to Ampicillin 32 (94.1%) and Nitrofurantoin 32(94.1%) followed by Trimethoprim/Sulfamethoxazole 25 (73.5%). In this study *Pseudomonas aeruginosa* showed 11 (100%) resistance to Ampicillin, Cefazolin and Ampicillin/Sulbactam. similar results by Islam et al., (2011) from Pakistan reported that *Pseudomonas aeruginosa* was (100%) resistance to Ampicillin, on the other hand *Pseudomonas aeruginosa* was (100%) sensitive to Ciprofloxacin.

Furthermore Sharma et al., (2005) from India reported that Ciprofloxacin was the most effective drug for *Pseudomonas aeruginosa* which similar to our finding. Although the emergence of antimicrobial
resistance is invariably associated with antimicrobial use, the multiple mechanisms of resistance, the frequency of gene exchange in the natural environment, and the nonspecific nature of many resistance mechanisms make developing resistance-specific strategies to reduce individual resistance phenotypes complicated and fraught with potential deleterious unintended consequences. Efforts to reduce overall antimicrobial exposure, for example, through organized efforts to identify appropriate minimal lengths of therapy, hold greater promise for reducing the burden of resistance. When a patient does not benefit from antimicrobial therapy chosen on the basis of clinical presentation, additional investigations are needed to determine the etiologic agent or exclude noninfectious diagnoses. To optimize microbiological diagnosis, clinicians should ensure that diagnostic specimens are properly obtained and promptly submitted to the microbiology laboratory, preferably before the institution of antimicrobial therapy (Leekha et al., 2011).

**Table 2. Frequency of microorganism’s positive culture from Throat infection**

<table>
<thead>
<tr>
<th>Isolated pathogens</th>
<th>Total No. and % of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>11</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>10</td>
</tr>
<tr>
<td>Sphingomonas paucimobilis</td>
<td>6</td>
</tr>
<tr>
<td>Enterobacter hormaechei</td>
<td>3</td>
</tr>
<tr>
<td>Coronobacter dublinensis ssp dublinensis</td>
<td>3</td>
</tr>
<tr>
<td>Pseudomonas luteola</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>34</td>
</tr>
</tbody>
</table>

**Table 3. The number and percentage of antibiotics resistance in Gram negative bacteria**

<table>
<thead>
<tr>
<th>Total No. of isolated bacteria</th>
<th>AM</th>
<th>AM/sul</th>
<th>PIP</th>
<th>CZ</th>
<th>CX</th>
<th>CA Z</th>
<th>CR O</th>
<th>IMP</th>
<th>M ER</th>
<th>AK</th>
<th>G M</th>
<th>T OB</th>
<th>CIP</th>
<th>F</th>
<th>SX T</th>
<th>CM</th>
<th>Le vo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas aeruginosa (11)</td>
<td>11</td>
<td>100%</td>
<td>11</td>
<td>100%</td>
<td>11</td>
<td>100%</td>
<td>1</td>
<td>9</td>
<td>9</td>
<td>81.8%</td>
<td>3</td>
<td>27.2%</td>
<td>1</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Proteus mirabilis (10)</td>
<td>10</td>
<td>100%</td>
<td>1</td>
<td>10%</td>
<td>2</td>
<td>10%</td>
<td>7</td>
<td>70%</td>
<td>1</td>
<td>10%</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>70%</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Sphingomonas paucimobilis (6)</td>
<td>5</td>
<td>83.3%</td>
<td>5</td>
<td>16.6%</td>
<td>5</td>
<td>83.3%</td>
<td>5</td>
<td>16.6%</td>
<td>5</td>
<td>83.3%</td>
<td>5</td>
<td>83.3%</td>
<td>5</td>
<td>16.6%</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Coronobacter dublinensis ssp dublinensis (3)</td>
<td>3</td>
<td>100%</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>66.6%</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>100%</td>
<td>1</td>
<td>33.3%</td>
<td>3</td>
<td>100%</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Enterobacter hormaechei (3)</td>
<td>3</td>
<td>100%</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>100%</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>100%</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>100%</td>
<td>2</td>
<td>66.6%</td>
<td>0</td>
</tr>
<tr>
<td>Pseudomonas luteola (1)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>100%</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Ampicillin (Am), Ampicillin / sulbactam (Am/sul), Piperacillin (PIP), Cefazolin (CZ), Cefazidine (CAZ), Ceftriaxone (CRO), Imipenem (IMP), Meropenem (MER), Amikacin (AK), Gentamicin (GM), Tobramycin (TOB), Ciprofloxacin (CIP), Nitrofurantoin (F), Trimethoprim-sulfamethoxazole (SXT), Cefixime (CFM), Levofloxacin (Levo).
The result represent ESBL production occurred in 26 (76.5%) out of (34) of Gram-negative bacteria isolates with highest incidence (100%) in
Coronabacter dublinensis ssp. dublinensis, Pseudomonas luteola for each, followed by Proteus mirabilis (90%), Pseudomonas aeruginosa (81.8%).
Comparing the results to other studies we notice similar finding has been obtained by Al-Haidari (2010) from Erbil (Iraq) who found that (76.3%)
of Gram-negative bacteria isolates were ESBL producers. Our results also were in agreement with Samatha and Parveen (2011) from India in which
found among (292) isolates of Gram-negative bacteria that have been taken from tertiary care hospital 201(69%) isolates were produced ESBL and showed that ESBL production ranged among Pseudomonas spp. (60%) and Proteus spp. (50%) in which it was lower than our results. Also Sanguinetti et al., (2003) from Italy reported that were (62.5%) ESBL positive among Enterobacteriaceae isolates. The ESBL producing organisms are a breed of multidrug resistant

### Table 4: Frequency of ESBL, AmpC β-lactamase and Metallo β-lactamase producing Gram-negative bacteria

<table>
<thead>
<tr>
<th>Gram-negative bacteria isolated</th>
<th>Total No. of isolated</th>
<th>No. &amp; % of isolated with ESBL</th>
<th>No. &amp; % of isolated with AmpC β-lactamase</th>
<th>No. &amp; % of isolated with Metallo β-lactamase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>11</td>
<td>Positive 9 (81.8%) Negative 2 (18.2%)</td>
<td>Positive 3 (27.3%) Negative 8 (72.7%)</td>
<td>Positive 7 (63.6%) Negative 4 (36.4%)</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>10</td>
<td>Positive 9 (90%) Negative 1 (10%)</td>
<td>Positive 4 (40%) Negative 6 (60%)</td>
<td>Positive 6 (60%) Negative 4 (40%)</td>
</tr>
<tr>
<td>Sphingomonas paucimobilis</td>
<td>6</td>
<td>Positive 2 (33.3%) Negative 4 (66.7%)</td>
<td>Positive 3 (50%) Negative 3 (50%)</td>
<td>Positive 5 (83%) Negative 1 (16.7%)</td>
</tr>
<tr>
<td>Coronabacter dublinensis ssp. dublinensis</td>
<td>3</td>
<td>Positive 3 (100%) Negative 0</td>
<td>Positive 0 Negative 3 (100%)</td>
<td>Positive 1 (33.3%) Negative 2 (66.7%)</td>
</tr>
<tr>
<td>Enterobacter hormaechei</td>
<td>3</td>
<td>Positive 2 (66.7%) Negative 1 (33.3%)</td>
<td>Positive 1 (33.3%) Negative 2 (66.7%)</td>
<td>Positive 3 (100%) Negative 0</td>
</tr>
<tr>
<td>Pseudomonas luteola</td>
<td>1</td>
<td>Positive 1 (100%) Negative 0</td>
<td>Positive 0 Negative 1 (100%)</td>
<td>Positive 1 (100%) Negative 0</td>
</tr>
<tr>
<td>Total</td>
<td>34</td>
<td>Positive 26 (76.5%) Negative 8 (23.5%)</td>
<td>Positive 11 (32.4%) Negative 23 (67.6%)</td>
<td>Positive 23 (67.6%) Negative 11 (32.4%)</td>
</tr>
</tbody>
</table>

As shown in table (4) thirty four isolates of Gram-negative bacteria were screened for ESBL production by double disk diffusion method (figure 2) of them 26 (76.5%) isolates were ESBL positive while 8 (23.5%) isolates were negative. The highest percentage of ESBL production occurred in Coronabacter dublinensis dublinensis, Pseudomonas luteola 100% for each followed by Proteus mirabilis 10 (90%), Pseudomonas aeruginosa 11(81.8%). Thirty four isolates of Gram-negative bacteria were screened for AmpC β-lactamase production by disk antagonism test figure (3). Of these 11 (32.4%) isolates were AmpC β-lactamase positive, while 23(67.6%) isolates were negative. The highest percentage of AmpC β-lactamase production occurred in Sphingomonas paucimobilis 6 (50%) followed by Enterobacter hormaechei 3 (33.3%) and Pseudomonas aeruginosa 11(27.3%). Thirty four isolates of Gram-negative bacteria were screened for Metallo β-lactamase production by Imipenem-EDTA combined disc test figure (4). of these 23 (67.6%) isolates were Metallo β-lactamase positive while 11 (32.4%) isolates were negative. The highest percentage occurred in Enterobacter hormaechei, Pseudomonas luteola 100% for each followed by Sphingomonas paucimobilis 6 (83.3%) and Pseudomonas aeruginosa 11 (63.6%).

One of the most important resistant mechanisms in Gram-negative bacteria against β-lactam antibiotics is induced by production of β-lactamases enzymes. The new broad-spectrum antibiotics such as Cephalosporins used in treatment of bacterial infections has led to the production of a new Class of broad-spectrum enzymes called β-lactamase (Yazdi et al., 2012). This part of the study was performed to assess the effect of types of β-lactamase production on the bacteria isolated from throat infection

Extended-spectrum β-lactamases are plasmid mediated β-lactamases which have the ability to hydrolyze β-lactam antibiotics containing an oxyimino group (e.g Cefazidime, Ceftriaxon, Cefotaxime or Aztreonam) (Paterson, 2000). Production of ESBL has been reported in virtually all species of Enterobacteriaceae (Silva Dias, et al., 2007).
pathogens that are increasing rapidly and becoming a major problem in the area of infectious diseases (Umadevi et al., 2011).

**Figure 2.** Double-disk diffusion test used for the detection of ESBL production in Gram negative bacteria; (A) ESBL positive, (B) ESBL negative

**Figure 3.** Disk antagonism test for the detection of AmpC β-lactamase production in Gram negative bacteria; (A) AmpC β-lactamase negative (Absence of blunting indicates negative), (B) AmpC β-lactamase positive (Blunting of the Ceftazidime disc adjacent to the Cefoxitin disk, positive)

**Figure 4.** Imipenem (IMP) - EDTA combined disc test used for the detection of Metallo β-lactamase production in Gram negative bacteria; (A) Metallo β-lactamase negative, (B) Metallo β-lactamase positive (IMP-EDTA increase clear zone of inhibition)

Clinical microbiological laboratories still face significant problems with ESBL screening and identification of ESBL pathogens can present with variations in vitro pattern of resistance to β-lactam agents. Proficiency testing studies performed by the World Health Organization and the Centers for Disease Control have raised concerns about the current ability of many clinical Laboratories to detect ESBL producing microorganisms (Al-Zarouni et al., 2008).

On the other hand the results in the present study also showed that out of (34) Gram-negative bacteria 11 (32.4%) isolates were produce AmpC β-lactamase and the highest percentage of AmpC β-lactamase was in *Sphingomonas paucimobilis* (50%) followed by *Proteus mirabilis* (40%) and *Pseudomonas aeruginosa* (27.3%).
Our results were similar to the finding of Samatha and Praveen, (2011) from India who showed that among Gram-negative bacteria (24.6%) were positive to AmpC, (33.3%) of them were Pseudomonas spp. which is similar to our finding and our finding for Proteus mirabilis that produce AmpC β-lactamase was similar to that reported by Ahmed, (2013).

Both ESBL and AmpC β-lactamase are encoded by plasmids and confer a selective advantage to strains harbouring these in a hospital setting (Taneja et al., 2008).

Detection of AmpC production in pathogens might be important for ensuring effective antibiotic therapy since the presence of an AmpC β-lactamase frequently seems to result in therapeutic failure when broad-spectrum Cephalosporins are used (Polsfuss et al., 2011).

Plasmid mediated AmpC β-lactamase represent a new threat since they confer resistance to Cephamycins and are not affected by β-lactamase inhibitors and can with loss of outer membrane porins provide resistance to Carbapenems, distinguishing between organisms has epidemiological significance and has therapeutic importance as well (Parveen et al., 2010).

Metallo β-lactamase is resistance determinants of increasing clinical relevance in Gram-negative bacteria. Because of their broad range, potent Carbapenemase activity and resistance to inhibitors, these enzymes can confer resistance to almost all β-lactams (Cornaglia et al., 2011).

Furthermore the results in the present study showed that out of (34) Gram-negative tested bacterial 23 (67%) isolates were produce MBL, the highest percentage of MBL producing was occurred in Corobacter dublinensis spp. dublinensis (66.7%) followed by Proteus mirabilis (40%) and Pseudomonas aeroginosa (36.4%), our results were similar to Ahmed, (2013) in which reported that out of (73) Gram-negative bacteria isolated from vaginal swabs 25 (34.2%) isolates produced MBL.

Also Peleg et al., (2005) from Australia reported that over (30%) MBL carrying isolates were predominately Enterobacteriaceae.

Resistance to Carbapenem is due to decreased outer membrane permeability, increased efflux systems, alteration of Penicillin binding proteins and Carbapenem hydrolyzing enzymes-Carbapenemase. These Carbapenemase are class B MBL (Varaiya et al., 2008).

The awareness of the existence of MBL initializes indication for the need for proper use of antibiotics and spread of multidrug resistance bacterial strains within these hospital and communities.

REFERENCES


