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Detection of Extended Spectrum Beta Lactamase (ESBL) Producing *Klebsiella pneumoniae* Associated with Tuberculosis Suspected Patients in Basra Governorate, South of Iraq

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Abstract **Objective**: To investigate extended spectrum β- lactamase (ESBL) producing Klebsiella pneumoniae isolated from sputum of tuberculosis suspected patients in Basra governorate. **Methods:** A total of 28 (30.4 %) isolates of K. pneumoniae were recovered from 92 sputum clinical specimens at Pulmonary and Respiratory Diseases Center (PRDC) in Basra Governorate, Iraq. All these isolates were tested for ESBL production by using chromogenic media. **Results:** Of 28 isolates of K. pneumoniae, 6 (21.4%) were positive for ESBL production. **Conclusion:** This finding demonstrates a high percentage of ESBL producers among clinical isolates of K. pneumoniae. Presence of ESBL producing K. pneumoniae associated with TB patient gives a high risk factor to patients.

Keywords: *K. pneumoniae, ESBL, Multidrug resistance, TB, chromoagar*

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1. Introduction

Klebsiella pneumoniae is a Gram-negative, non-motile, encapsulated, lactose fermenting, facultative anaerobic, rod shaped bacterium found in the normal flora of the mouth, skin and intestines [1].

It is an important bacterial pathogen in humans that is commonly associated with opportunistic and hospital-associated infections. Increasing levels of multiple-antibiotic resistance associated with this species pose a major emerging clinical problem [2].

The beta-lactam group of antibiotics is the largest and most commonly used group globally. It consists mainly of penicillins, cephalosporins, monobactams, carbapenems, and cephamycins, which are semi-synthetic compounds originating from fungi and bacteria [3].

Beta-lactamases are enzymes located in the periplasm on the outer surface of the inner membrane of the cell envelope in Gram-negative bacteria [4].

In 1983, the first report of plasmid-mediated beta-lactamases capable of hydrolyzing extended-spectrum cephalosporins was published. They were named extended-spectrum beta lactamases (ESBLs) and they have since been described worldwide [5].

In the ever evolving world of bacterial resistance to antibiotics, one of the more frequent and powerful weapons of bacteria is the production of beta-lactamases. In Gram-negatives this is the most important way of bacterial resistance against beta-lactam antibiotics.

The emergence of plasmid-encoded ESBLs is a significant evolution in antimicrobial resistance. Outbreaks due to the dissemination of ESBL-producing bacterial strains and to the dissemination of ESBL-encoding plasmids among different species of the family Enterobacteriaceae have been described in hospitals and other health care facilities [6,7].

Many of the emerging antimicrobial resistance problems of the nineties have been characterized by difficulty in the recognition of resistance in the laboratory, particularly by rapid susceptibility-test methods [8].

There are many laboratory methods for detection of ESBL. One of these methods use detective and selective media like Chromogenic media, which is new media for identification of many bacterial species, It's a rapid test with high sensitivity and specificity.

Chromogenic media have many advantages, one of its, needles to further biochemical test in microorganism identification [9].

The study aimed to detect extended spectrum β-lactamase ESBL producing *K. pneumoniae* isolated from sputum of tuberculosis(TB)suspected patients in Basra governorate by using chromoginic media.

2. Material and Methods

2.1. Samples Collections

A total of 92 (49 male 53.2%)(43 female 46.7%) of sputum of TB suspected patients collected from Pulmonary and Respiratory Diseases Center (PRDC) in Basra, Iraq, during March to May 2013. Ten ml sputum were collected from each persons in the early morning and directly sputum cultured in MacConkey agar for the growth of *K. pneumonia* and incubated aerobically at 37 °C overnight.

2.2. Isolation and Identification of *K. pneumonaie*

K. pneumoniae identification was performed with microbiological methods and conventional biochemical tests.

2.3. Chromogenic Culture for ESBL

All positive samples (*K. pneumoniae*) have been inoculated on CHROMagar Orientation (CHROMagar™, Paris, France) with supplement, then incubated aerobically at 37°C for 18 to 24 h. Appearance of coloured colonies on the chromogenic medium was considered a positive test result.

3. Results

Of ninety two patients with suspected TB attended the PRDC who were suffering from upper respiratory tract infections, twenty eight samples (30.4%) were Gram-negative, short rod-shaped bacterium, plump, straight rods, non-motile, encapsulated, facultative anaerobic, large, dome-shaped, mucoid colonies on blood agar, and lactose fermenting colonies on MaCconkey agar. Negative indole test, negative methyl red test, positive Voges-Proskauer test, positive citrate-utilization test, positive urease test, acid and abundant gas production from glucose, lactose, sucrose, maltose and mannitol sugar fermentation tests, categorized these isolates as *K. pneumoniae*. All of these inoculated on ESBL CHROMagar, only 6(21.4%) gave positive ESBL. The results are summarized in Table 1 and Table 2.

Table 1. Summarized results of Ninety two samples

No. of Isolates	Sex		Age (Yr)	Previous TB	Positive TB Smear AFB	Positive *K. Pneumoniae*	Positive ESBL
	M	F					
92	49	43	15-77	16(17.3%)	31(33.7%)	28(30.4%)	6(21.4%)

Table 2. Identified of ESBL by chromogenic medium

Isolate No.	Positive *K. Pneumoniae* (Biochemical) 28	Positive ESBL 6	Colonies appearance on Chromoagar plates
2	+	-	Colourless colonies
4	+	+	Metallic blue colonies (1-3mm)
7	+	+	Metallic blue colonies (1-3mm)
8	+	+	Metallic blue colonies (1-2mm)
9	+	-	Pink colonies
10	+	-	Colourless colonies
12	+	+	Metallic blue colonies (1-2mm)
13	+	-	Colourless colonies
17	+	-	Colourless colonies
19	+	-	Colourless colonies
20	+	-	Brown colonies
28	+	-	Colourless colonies
31	+	+	Metallic blue colonies (1-3mm)
32	+	-	Colourless colonies
37	+	-	Colourless colonies
40	+	-	Brown colonies
49	+	-	Colourless colonies
52	+	-	Colourless colonies
58	+	-	Colourless colonies
59	+	-	Colourless colonies
66	+	-	Colourless colonies
67	+	-	Colourless colonies
77	+	-	Colourless colonies
78	+	+	Metallic blue colonies (1-3mm)
79	+	-	Inhibited
82	+	-	Colourless colonies
87	+	-	Brown colonies
89	+	-	Colourless colonies

4. Discusstion

Amongst antibiotics, ß-lactams are the safest and the most widely used antibiotics to date [10].

The extended spectrum ß-lactams are commonly used empirically for the treatment of Gram-negative sepsis. But the emergence of ESBL producing organisms has posed a serious threat for their continuing use[11].

Mortality, morbidity and cost of treatment have considerably risen because of these resistant isolates [12].

The emergence of plasmid-encoded ESBLs is a significant evolution in antimicrobial resistance [8], and this result agree with our study.

In the current study, 6 out of 28 isolates (30.4%) were of ESBL-producing *K. pneumoniae* by using chromogenic medium.

Using chromogenic media is one of the rapid diagnostic methods that is introduced as appropriate alternative for conventional method in developed countries. Applying these sensitive, accurate and specific methods in diagnosis process is a turning point in analytical microbiology and considered as powerful tools [13].

Utilizing these media can eliminate necessity of further subculture and biochemical test in identification process of bacteria. These technique are based on using substrate material for specific microorganism enzyme, and according to the produced colour, the microorganism can be identified easily [14]

Laboratory-based detection of ESBL producers from clinical specimens is highly sensitive and specific, combined with a short reporting time for results, thus decreasing the workload and reducing the need for unnecessary confirmatory tests [15].

ESBL-producing bacteria are often associated with multi-drug resistance, i.e. resistance to other classes of drugs like quinolones, aminoglycosides and cotrimoxazole [16].

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