

## Gram negative bacilli produces extended spectrum beta-lactamases isolated from diabetic foot wound infection

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### Abstract:

ESBL producing Gram Negative bacteria have emerged as a major threat worldwide as they produce the enzyme Beta-lactamase which hydrolyse beta-lactam antibiotics containing an oxyimino group (Third generation cephalosporins and aztreonam) and are inhibited by -lactamase inhibitors such as clavulanic acid, sulbactam and tazobactam. A total of 32 isolates from diabetic foot wounds of patients attended to outpatient clinics in the central hospital of Misurata city over a period of 3 months were included in the study. Bacterial species were identified by using standard microbiological culture and biochemical reactions, while screening and double-disc synergy confirmatory tests were used to detect ESBLs production. This study finding showed *Pseudomonas aeruginosa* (*p. aeruginosa*) was the dominant gram negative bacterial species (11/32) isolated from diabetic foot wounds. Moreover, study results reveal low prevalence of ESBLs producing isolates among included bacterial collection (1/32; 3%) whereas, positive isolate was identified as *p. aeruginosa*.

### Introduction:

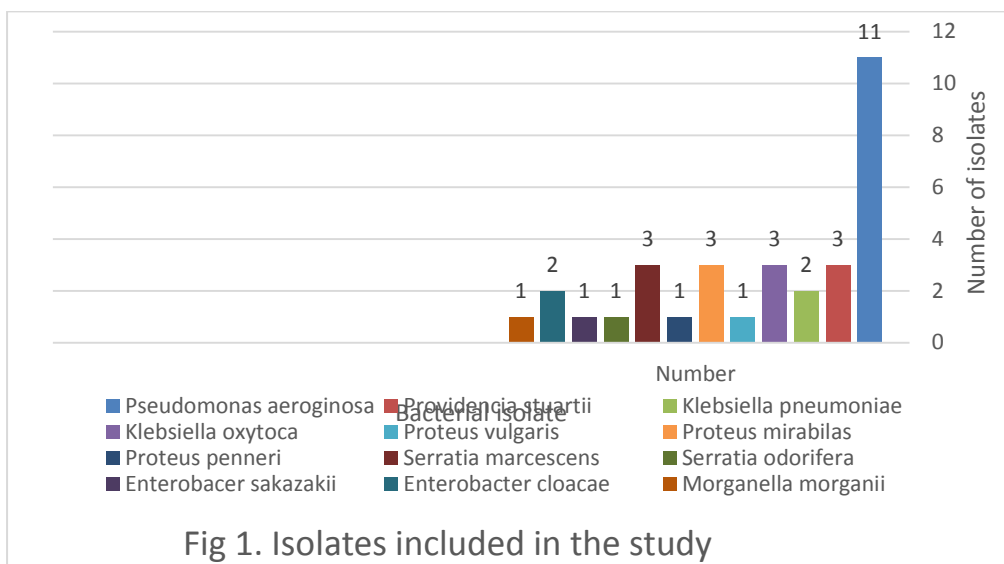
The first beta-lactamase was identified in *Escherichia coli* (*E. coli*) prior to the release of penicillin for use in medical practice [1]. In Gram negative pathogens, beta-lactamase production remains the most important contributing factor to Beta-lactam resistance [2]. The four major groups of beta-lactams penicillin, cephalosporins, monobactams and carbapenems have a beta-lactam ring which can be hydrolysed by beta-lactamases resulting in microbiologically ineffective compounds [3]. The persistent exposure of bacterial strains to a multitude of beta-lactams has led to overproduction and mutation of beta-lactamases. These beta-lactamases are now capable of hydrolyzing penicillin, broad-spectrum cephalosporins and monobactams. Thus, these are new beta-lactamases and are called as extended spectrum beta lactamases (ESBLs) [4]. The first plasmid mediated beta-lactamase was described in early 1960. ESBLs have been isolated from a wide variety of

Enterobacteriaceae, *Pseudomonas aeruginosa* and *Capnocytophaga ochracea* [5-7]. Previous studies showed prevalence of gram negative bacilli in hospitals and different environments [8]. In India, study showed prevalence of ESBL producing *Klebsiella pneumonia* was higher in hospital isolates compared to community collection [9]. Studies conducted in Africa reported high prevalence of ESBLs among gram negative species [10,11]. Detection of ESBLs in gram negative bacilli rather than Enterobacteriaceae were confirmed, whereas, extended-spectrum beta-lactamase-producing *Salmonella enterica* was found in Algeria [12] and others reported ESBLs positive *Salmonella Typhimurium* isolates obtained from food samples in the Netherlands [13]. Emergence of multidrug-resistant (resistant to beta-lactams, aminoglycosides, and quinolones) *P. aeruginosa* strains causes a serious problem in tertiary Greek hospital [14]. Although intrinsically sensitive to  $\beta$ -lactams (e.g., Ceftazidime [CAZ] and Imipenem [IPM]), aminoglycosides (e.g., Amikacin [AMK] and Tobramycin), and fluoroquinolones (e.g., Ciprofloxacin [CIP] and Ofloxacin [OFX]), *P. aeruginosa* resistant to these antibiotics has emerged and is widespread [15]. Carbapenems are the drugs of choice for the treatment of infections caused by multi-resistant gram-negative bacilli [16]. An increasing prevalence of carbapenem resistance mediated by acquired metallo- $\beta$ -lactamases (MBLs) is being reported, particularly for *P. aeruginosa* clinical isolates in several countries [17]. To our knowledge there is no previous published or reviewed studies have been performed on investigation of ESBLs prevalence among of gram negative bacilli clinical isolates in Misurata city. This study conducted to investigate the prevalence of ESBLs among gram negative bacilli isolates obtained from diabetic foot wound of patients attended to clinics of central hospital of Misurata city. The study was conducted from 1<sup>st</sup> of June to 30<sup>th</sup> of August 2016.

## Materials and Methods

### Isolates collection and bacterial identification

Using cotton swabs, thirty-two isolates of gram negative bacilli of different bacterial species (Fig.1) were collected from diabetic foot wounds of forty patients at outpatient clinics of central hospital of Misurata city. Collected isolates were identified by using phenotypic characters of pure isolate grown on bacterial cultures, biochemical tests and confirmed by API 20E biochemical test (bioMérieux's API®).



### Sample processing

Antimicrobial susceptibility and detection of ESBL was performed according to CLSI guidelines [18]. Included isolates were identified and stored at  $-20^{\circ}\text{C}$  for further tests. Frozen bacterial species were cultured on blood agar and pure isolated colonies inoculated on peptone water and turbidity was adjusted to 0.5 Mc Farlands standard prior of antimicrobial susceptibility, screening of ESBL and Double-Disc Synergy Test (DDST).

### ESBL-Producing Isolates

#### Screening test

According to CLSI guidelines, strains showing zone of inhibition of  $\leq 22\text{mm}$  for ceftazidime,  $\leq 27\text{mm}$  for cefotaxime, and  $\leq 25\text{mm}$  for ceftriaxone were selected for conformational tests of ESBL.

#### Confirmatory test: Double-Disc Synergy Test

Colonies showed positive result for ESBL screening test included in the confirmatory test (DDST). Amoxicillin clavulanic acid disc ( $20/10\ \mu\text{g}$ ) was placed in the center of plate. Both side of Amoxicillin/ clavulanic acid disc, a disc of ceftriaxone ( $30\ \mu\text{g}$ ) and ceftazidime ( $30\ \mu\text{g}$ ), were placed with center to center distance of 20mm to centrally placed disc. The plate was incubated at  $37^{\circ}\text{C}$  overnight. ESBL production was interpreted as positive when the inhibition zone potentiated toward the central disc containing clavulanic acid.

## Results and Discussion

Study findings showed 32 isolates were positive for ESBLs production in screening test while only one isolate identified as ESBLs producers in the confirmatory test. In Libya, studies conducted in Zleten and El Khoms cities found 13.4% of *E. coli* isolate obtained from pediatric fecal samples were producing ESBL [19]. In specimens collected from inpatients and outpatient clinics of Trauma and Surgery Departments in Tripoli Central Hospital, the prevalence of ESBLs among 383 of *E. coli* were 8.6% and 15.5% of 209 *Klebsiella pneumonia*. Production of ESBL among isolates obtained from inpatient clinics were significantly higher than others collected from outpatient clinics [20].

Low prevalence of ESBLs positive isolates in this study (1/32; 3%) was contradicted to other study results that showed high prevalence of producing bacterial species [9,11]. These studies (above mentioned) included clinical bacterial isolates obtained from patients admitted at hospitals which lead to high exposure to the antimicrobials and preloading to high antimicrobial resistance. In agreement with our study finding, a study included bacterial isolates from outpatient and inpatient clinics reveals ESBLs bacterial isolates were significantly higher ( $P < 0.000001$ ) in inpatient group. Isolates included in our study collected from patients attended to outpatient clinics, thus exposed to the out-hospital environment (community) and have less exposure to the antimicrobials.

This study showed one isolate identified as ESBLs producer and identified as *P. aeruginosa*. Other Studies included bacterial collection from patients admitted at hospitals showed most ESBLs positive bacterial isolates identified as *K. pneumonia* and *E. coli* [9,11].

The results revealed the dominant gram negative bacterial species isolated from foot-diabetic wound was *P. aeruginosa*, also ESBLs production was low prevalent (3%) and detected in one isolate of *P. aeruginosa*. Further studies are needed to detect ESBLs production among different pathogenic bacterial species prevalent in hospitals and communities

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