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Phenotypic detection of extended spectrum beta-lactamases (ESBLs) in Gram-negative bacilli isolated from deferent clinical source

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Abstract— This study aimed to the detection of extended spectrum beta-lactamases by phenotypic and genotypic methods in nosocomial Gram-negative isolates. A number of 194 samples from hospitalized patients (urine, burn swab, surgical swab, and sputum) were collected These patients were at Al-Hussein and Al-Nasiriyah teaching hospitals in Al-Nasiriyah city. ESBLs enzymes were detected phenotypically by ceftazidime (30 µg) and cefotaxime (30 µg) (Screening test) and Double Disk Synergy Test (DDST) (Confirming test). The results showed that out of 109 isolates 89 (81.65%) were Gram negative bacilli. A total of 68 Gram-negative were β-lactamases producer. Moreover, the results of screening test recorded that 55 (80.88%) of isolates were resistant to cefotaxime and ceftazidime, while 17 (25%) isolates produced ESBLs by (DDST).

Keywords— Extended-spectrum β-lactamases, Gram-negative bacilli, DDST

I. Introduction

Most β-lactam antibiotics, including cephalosporins, penicillins, aztreonam, and related oxyimino β-lactams, can be hydrolyzed by extended-spectrum β-lactamases, with the exception of cephamycins (cefoxitin) and carbapenems, which can only be blocked by β-lactamase inhibitors such clavulanic acid (Gebremichael *et al.*, 2020). (ESBLs) have been found in almost Gram-negative bacteria, especially Enterobacteriaceae and P. aeruginosa (Fanaei *et al.*, 2021). These enzymes are include members of the TEM, SHV, CTX-M, and OXA enzyme families (Reygaert *et al.*, 2018). The property of ESBLs, which are active site serine Ambler's class A or class D β-lactamases, to hydrolyze and produce resistance to third- and fourth-generation cephalosporins and monobactams, is one of their distinguishing features (Hamadamin *et al.*, 2019)

In the recent two decades, numerous substantial alterations in ESBL-producing isolates have been observed globally. ESBL producers have emerged as an important multidrug-resistant pathogen (Shilpakar *et al.*, 2021). Since these compounds were identified in the early 1980s, they have become prevalent in Enterobacteriaceae isolated from both hospital-associated illnesses and community-acquired infections (Castanheira *et al.*, 2021). The most often implicated pathogens in large nosocomial outbreaks produced by ESBL-producing Gram-negative bacilli are *E. coli* and *K. pneumonia (.Manzur et al., 2007)* The ESBL enzyme genes are often present in plasmids. They also are available in chromosomal DNA (Guiral *et al.*, 2018).

II. Materials and Methods

Collection of Samples

Between December 2021 and March 2022, a total of 194 samples were collected from hospitalized patients (including urinary tract 75, surgical wound 52, sputum from respiratory tract 40 and burn swabs 27). These samples were taken from patients at Al-Hussein and Al-Nasiriyah teaching hospitals. All samples were transported to the laboratory and inoculated on blood agar and MacConkey agar and incubated at 37 ˚C for 24 to 48 hr. The identification of bacteria was dependent on the morphological examination, microscopic examination, biochemical tests and confirmed by API 20 E system and VITEK-2 system.

Detection of β-lactamase production

All isolates were tested for their ability to the production of β-lactamases by Rapid iodometric method according to Miles *et al.*, 1994 (Hekma *et al.*, 2020).

phenotypic methods for detection Extended-spectrum β-lactamases (ESBLs) production

1-Screening test of ESBLs

To screen all isolates for the production of ESBL enzymes, two discs were placed on Muller Hinton Agar with the test bacterium were ceftazidime (30 µg) and cefotaxime (30 µg) at a distance of 30 mm (center to center) and incubation at 37˚C for 24hr according to the CLSI guidelines (CLSI 2021), if the diameter of inhibition zone around ceftazidime is ≤ 22 mm or Cefotaxime zone ≤ 27 mm it was considered positive.

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2-Confirmation of ESBL production

In this part of the present study, ESBLs production was confirmed by Double Disk Synergy Test (DDST). On Muller Hinton Agar with suspended bacteria was incubated with cefepime (30 µg), aztreonam (30 µg), ceftazidime (30 µg), cefotaxime (30 µg), and ceftriaxone (30 µg) alone with Augmentin (amoxicillin 20 µg, clavulanate acid 10 µg) at the center and 30 mm apart (center to center) on an inoculated agar plate. A clear extension of the edge of the cephalosporins disk inhibition zone toward the disk containing clavulanate was interpreted as synergy, indicating the presence of an ESBL (Thomson *et al.*, 2013).

III. RESULTS

Out of 194 hospitalized patients, 109 bacterial isolates were recorded as positive cultures with a percentage of 56.19%, urinary tract specimens registered a high number of positive cultures was recorded, it was 47 (43.12%). The results appeared that the burn swabs were the lower specimens that gave positive culture 11 (10.09%). With the remaining 85 with a percentage 43.81% being negative growth, 75 (38.66%) of samples were collected from the Urinary tract, 52 (26.80%) from the surgical wound, 40 (20.62%) respiratory tract and 27 (13.92%) burn swabs.as shown in Table 1.

Urinary tract infections were the most frequent source of the isolated Gram-negative bacteria (38 (42.70%)), followed by surgical wounds (23 (25.84%), respiratory tract (19 (21.35%), and 9 (10.11%) of burn swabs, out of the 109 positive cultures from hospitalized patients, the results found that Gram-positive bacteria registered 20 (18.35%)) isolates identified from nosocomial infection (SEE table 2).

Total of 89 isolates of Gram-negative bacilli were tested for their ability to the β-lactamase enzyme by the rapid iodometric method. The results showed that 68 of isolates revealed positive results of β-lactamase production. *E. coli* recorded the high percentage 26 (38.24%) following by *K. pneumoniae* 17 (25.00%) as demonstrated in Table 3.

Production of Extended Spectrum lactamases (ESBL)

The ESBL initial screen disk test was assessed using cefotaxime and ceftazidime disks. A number of 68 isolates1of Gram negative were screened and verified for ESBL production. The results of the study showed that 55 (80.88%) of isolates were resistant to cefotaxime and ceftazidime and ESBL positive during the initial screening and 17 (25%) isolates producing ESBLs, with confirmatory test (DDST).(Table 4)

Table 1: Numbers and percentages of nosocomial infections (IN) from different sites of hospitalized patients.

Site of NI	No. & (%) positive culture	No. & (%) negative culture	Total & (%)
Urinary tract	47 (43.12)	28 (32.94)	75 (38.66)
Surgical wound	28 (25.69)	24 (28.24)	52 (26.80)
Respiratory tract	23 (21.10)	17 (20.00)	40 (20.62)
Burn swabs	11 (10.09)	16 (18.82)	27 (13.92)
Total	109 (56.19)	85 (43.81)	194 (100.00)

Table 2: Numbers and percentages of Gram negative bacteria isolated from nosocomial infections.

Site of NI	No. and (%) of Gram-negative isolates	No. and (%) of Gram-positive isolates	Total
Urinary tract	38 (42.70)	9 (45.00)	47 (43.12)
Surgical wound	23 (25.84)	5 (25.00)	28 (25.69)
Respiratory tract	19 (21.35)	4 (20.00)	23 (21.10)
Burn swabs	9 (10.11)	2 (10.00)	11 (10.09)
Total	89 (81.65)	20 (18.35)	109 (100.00)

Table 3: Number and percentage of β-lactamase producing Gram-negative bacterial isolates by the rapid iodometric method.

Gram-negative bacilli	**No. of isolates**	**No. and (%) of β-lactamase producing isolates**
E. coli	35	26 (38.24)
K. pneumoniae	24	17 (25.00)
P. aeruginosa	19	15 (22.06)
A. baumannii	6	5 (7.35)
E. cloacae	5	5 (7.35)
Total	89	68

IV. Discussion

The results showed that, out of 68 β-lactamases 55 (80.88%) were ceftazidime and cefotaxime resistant. This high percentage of ESBLs producers agrees with the results of a study performed in India by Rajivgandhi *et al.*, (2018) were showed that (84%) of Gram-negative bacterial isolates from clinical specimens against cefotaxime and ceftazidime were potentially positive. This may be too extensive used for broad-spectrum antibiotics and to a higher level in the community setting, combined with a disregard for laboratory testing of clinical isolates' development of ESBL (Al-Ouqaili *et al.*, 2011).

Moreover, the results of the present study illustrated that the isolates were E. coli isolates are significantly more frequently for ESBL-producing strains by both phenotypic methods screening and confirmatory tests. In particular beyond the confines of a clearly defined epidemic, the mechanisms of transmission and dissemination of ESBL- E. coli in hospital settings are not entirely known. Although it has traditionally been thought of as the most likely mechanism of transmission (Adler *et al.*, 2012). This percentage agrees with the results of a study from Saudi Arabia by (Kader and Angamuthu, 2005) and with the Chinese study of Wang *et al.* (2003), In Klebsiella spp isolates, ESBL production was found in 23.64%. These results are in agreement with the Egyptian study of Ahmed *et al.*, (2013) that demonstrated that the ESBL rate in Klebsiella pneumoniae was 21% which isolated from hospitalized patients.

Table 4: Numbers and percentages of ESBLs producing Gram-negative bacterial isolates by phenotypic tests.

Source of sample	Bacteria	No. β-lactamase producer isolates	No. and (%) Suspected ESBL of producing Gram-negative isolates			
			Screening test		Confirmatory test	
			positive results	negative results	Positive results	Negative results
Hospitalized patients	*E. coli*	26	21 (38.18)	5 (38.46)	8 (47.06)	18 (35.29)
	K. pneumoniae	17	13 (23.64)	4 (30.77)	5 (29.41)	12 (23.53)
	P. aeruginosa	15	12 (21.82)	3 (23.08)	3 (17.65)	12 (23.53)
	E. cloacae	5	4 (7.27)	1 (7.69)	0 (0.00)	5 (9.80)
	A. baumannii	5	5 (9.09)	0 (0.00)	1 (5.88)	4 (7.84)
Total		68	55 (80.88)	13(19.12)	17(25.00)	58(75.00)

In the present study, the results revealed that 25% of Gram-negative isolates were ESBLs producers by DDST. These results are similar to the results of the study of nosocomial Gram-negative bacilli isolates by Singh *et al*. (2012) and Mshana, (2009) who found ESBLs producer was 27% and 29%, respectively. These results were lower than the results conducted by Saha *et al*. (2017) were registered 41.40% of the Gram-negative bacilli isolated from nosocomial infections. The use of clavulanic acid to inhibit an ESBL may induce high level expression of the chromosomal AmpC enzyme and may then antagonize rather than protect the antibacterial activity of the partner lactam, preventing the synergistic effect required to detect ESBL production, which may explain the lower percentage in this method (Apfalter *et al.*, 2007).

In concluded Gram-negative bacteria were the most pathogens causing nosocomial infections. *E. coli* and *K. pneumoniae* were the majority of Gram-negative isolates that infected hospitalized patients. *E. coli* and *K. pneumoniae* were the most isolates producing ESBLs.

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