

## Comparison of Double Disk Synergy Test and Combination Disk Test Methods for the Detection of Extended-Spectrum Beta-Lactamase Production among *Enterobacteriaceae*

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### Abstract

**Background:** The emergence and spread of Extended-spectrum beta-lactamases (ESBLs) producing *Enterobacteriaceae* are serious and expanding public health problems globally. Rapid and accurate detection of these bacteria facilitates infection prevention efforts in healthcare facilities. The aim of this study was to determine the levels of agreement between double disk synergy test (DDST) and combination disk test (CDT) for the detection of ESBL among *Enterobacteriaceae* isolated from different clinical specimens.

**Methods:** A total of 426 *Enterobacteriaceae* isolates were obtained from different clinical specimens from four clinical bacteriology laboratories. Fresh colonies of the isolates were recovered using MacConkey and 5% sheep blood agar plate. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method on Muller Hinton agar (MHA). All the isolates were screened for ESBLs production using cefotaxime and ceftazidime as per CLSI guideline. Each ESBLs specious isolates were confirmed by CDT and DDST. The overall percent agreement, positive percent agreement (PPA) and negative percent agreement (NPA) was calculated. Data was entered and analyzed using SPSS version 20.

**Results:** Using cefotaxime, 265 and ceftazidime, 259 were identified as potential ESBLs producing isolates. Of 265 screening ESBL positive *Enterobacteriaceae*, 92.8% (246/265) were confirmed as ESBLs producer by CDT, and 84.5% (224/265) by DDST. The most frequent *Enterobacteriaceae* were *E. coli* 228 (53.5%) and *K. pneumoniae* 103 (24.1%). The magnitude of ESBL was 246 (57.7%) by CDT and 224 (52.6%) by DDST. The highest frequencies of ESBL producers were found in blood specimen (84.4%), and from isolate in *K. pneumoniae* (85.4%). The overall percent agreement, positive percent agreement and negative percent agreement between DDST and CDT were 91.7%, 91.0 and 100%, respectively.

**Conclusion:** The DDST provided a very good agreement with the CDT for the detection of ESBL. Since DDST method can be performed together with AST, it allows immediate, accurate clinical decisions to prescribe appropriate antibiotics treatment or isolation of patients. Detection of ESBL in conjunction with the routine antibiotics susceptibility can help for infection control caused by such bacteria and timely treatment of a patient with best antibiotics.

**Keywords:** Extended-Spectrum-Beta-Lactamase; *Enterobacteriaceae*; Combination-Disk-Test; Double-Disk-Synergy-Test; Positive-Percent-Agreement; Negative-Percent-Agreement; Ethiopia

## Abbreviations

ESBLs: Extended-Spectrum Beta-Lactamases; DDST: Double Disk Synergy Test; CDT: Combination Disk Test; MHA: Muller Hinton Agar; PPA: Positive Percent Agreement; NPA: Negative Percent Agreement; EPHI: Ethiopian Public Health Institute; EHAO: Ethiopian National Accreditation Office; ICL: International Clinical Laboratories; TASH: Tikur Anbessa Specialized Hospital; YMCH: Yekatit 12 Medical College Hospital

## Introduction

Extended-Spectrum Beta-Lactamase are hydrolytic enzymes that mediate resistance to extended-spectrum penicillin, cephalosporins, and monobactams except for cephamycins, carbapenems, and beta-lactamase inhibitors like clavulanic acid and sulbactam. A worrisome increasing trend has been seen in the development of resistance to extended-spectrum cephalosporins, by ESBL producing *Enterobacteriaceae* [1-3]. Among *Enterobacteriaceae*, ESBL are found mostly in *Klebsiella* spp. and *E. coli*. Furthermore, it has been reported in other *Enterobacteriaceae* families, such as *Enterobacter* spps, *Proteus* spps, *Citrobacter* spps, *Morganella* spps, *Providencia* spps, *Salmonella* spp, and *Serratia* spps [4-6].

Being plasmid mediated, many ESBL-producing *Enterobacteriaceae* are also resistant to other commonly used antibiotics, namely, aminoglycosides, sulphonamides, and the fluoroquinolones [7,8]. Consequently, many patients impelled to take the 'last resort' antibiotics treatment such as carbapenems drugs [1,9]. However, the use of carbapenems has led to the rapid selection of carbapenem-resistant *Enterobacteriaceae* [10]. Only a few antibiotic such as carbapenems, colistin, tigecycline are available to treat infection caused by ESBL producing bacteria, although their *in vivo* efficacy and/or toxicity is not well known [11,12].

The most important mechanism of resistance to beta-lactam antibiotics involves the production of beta-lactamases, in particular ESBLs. Extended-spectrum beta-lactamases inactivate beta-lactam antibiotics and continues to be the prominent cause of  $\beta$ -lactam antibiotics resistance among *Enterobacteriaceae* worldwide. ESBLs producing *Enterobacteriaceae* are important members of antibiotic-resistant bacteria that cause hospital and community-acquired infections [3,13].

Many clinical laboratories including the wider medical community are not fully aware of the importance of ESBLs and how to detect them. The laboratories may also lack the resources to detect and to play role in the control of the spread of these resistance mechanisms [14,15]. This lack of understanding or resources is responsible for a continuing failure to respond appropriately to prevent the rapid worldwide dissemination of pathogens possessing these beta-lactamases. The consequence has been preventable treatment failures (sometimes fatal) in patients who received inappropriate antibiotics [16,17] and outbreaks of multidrug-resistant bacteria, *Enterobacteriaceae* that required expensive control efforts.

Detection of ESBLs which are being produced by *Enterobacteriaceae* remains a challenge for microbiologists. Many clinical laboratory professionals may not be fully aware of the importance of organisms producing ESBLs and their method of detection. Failure to detect accurately these enzymes has contributed to the uncontrolled spread, and sometimes to therapeutic failures in patients who received inappropriate antibiotics which resulted in outbreaks of multi-drug resistant [18,19]. In a clinical management scenario detecting significant isolate also means there is therapeutic resistance to all extended-spectrum cephalosporins (indeed, to all cephalosporins, aztreonam, and penicillins). Since many ESBL-producing bacteria are also resistant to other antimicrobial agents, the treatment of choice in such circumstances should be the use of the carbapenems or cephamycins, based on the sensitivity result. ESBLs detection is an essential interest for infection control and epidemiological program [20]. We compared two phenotypic methods, Combination Disk Test (CDT) and Double Disk Synergy Test (DDST) to indicate the use of simple, reliable and cost effective test method. Hence the aim of this study was determine the levels of agreement (that is, positive by both or negative by both) between double disk synergy test (DDST) and combination disk test (CDT) for the detection of ESBLs.

## Materials and Methods

This comparative cross-sectional study design was conducted from January to May 2017 at Ethiopian Public Health Institute (EPHI) Clinical Bacteriology and Mycology Laboratory in Addis Ababa. This laboratory is the national referral and research laboratory for the country. Furthermore, it is accredited by the Ethiopian National Accreditation office (ENAO) for its quality and competence. The *Enterobacteriaceae* isolates used for this study were collected from four bacteriology laboratories, namely EPHI Clinical Bacteriology and Mycology laboratory, bacteriology laboratory of International Clinical Laboratories (ICL), Tikur Anbessa Specialized Hospital (TASH) and Yekatit 12 Medical College Hospital (YMCH) bacteriology Laboratory.

A total of 426 consecutive non-repetitive *Enterobacteriaceae* isolates were collected from the four bacteriology laboratories. These isolates were identified from different clinical specimens; 272 from urine, 90 from blood 40 from pus, 11 from body fluids, 6 from sputum, 3 from ear discharge, 2 from eye discharge and 2 from cerebrospinal fluid (CSF). The isolates were collected using *Tryptone Soy Broth* (TSB) (Oxoid LTD, Basingstoke, Hampshire, England) containing 20% *Glycerol* and temporarily stored at -20°C in the respective laboratories. The isolates were transported to EPHI clinical bacteriological laboratory using a cold box with ice within a week after collection.

### **Culture and identification**

The isolates stored at freezer temperature were recovered by re-suspension of the stored isolate in *Tryptose Soy Broth* (Oxoid LTD, Basingstoke, Hampshire, England). After a few hours, it was inoculated and incubated on MacConkey agar (Oxoid LTD, Basingstoke, Hampshire, England) at 37°C for 18-24 hours. Following incubation, the colony was characterized by colony appearance, Gram stain and biochemical reaction. Pure isolate sub-cultured on to 5% sheep blood agar (HiMEDIA Laboratories Pvt. Ltd, Mumbai, India) were used to conduct antibiotic susceptibility test and confirmatory test for ESBLs production.

### **Preparation of clavulanate stock solution**

The combination disks (Ceftazidime-clavulanate (30 µg/10 µg) and cefotaxime-clavulanate (30 µg/10 µg) disks) utilized for CDT method were prepared from in-house made clavulanate solution according to CLSI guideline [21]. The stock clavulanate solution at 1000 µg/ml was prepared from potassium clavulanate powder (Sigma-Aldrich Corp, St. Louis, MO USA), and stored at -70°C freezer until it was applied on ceftazidime (30 µg) and cefotaxime (30 µg) antibiotic disk. During testing, 10 µL of clavulanate solution was added to ceftazidime (30 µg) and cefotaxime (30 µg) disks (Abtek Biologicals Ltd, Liverpool, United Kingdom), and allowed for about 30 minutes for the clavulanate solution to absorb and the disks to be dried enough for application. The prepared combination disks were used within 30 minutes after they get dried.

### **Antibiotic susceptibility testing**

Antibiotics susceptibility testing was carried out by the Kirby-Bauer disk diffusion method and the results were expressed as susceptible, intermediate or resistant according to CLSI guideline [21]. Muller-Hinton Agar (MHA) (Oxoid LTD, Basingstoke, Hampshire, England) plates were inoculated and antimicrobial disks were applied on the plate. The antibiotic disks used in this study to determine the susceptibility pattern of ESBLs producing *Enterobacteriaceae* were amoxicillin-clavulanic acid (AMC: 20/10 µg), cefotaxime (CTX: 30 µg), ceftazidime (CAZ: 30 µg), cefepime (FEP: 30 µg), Cefoxitin (30µg), meropenem (MER: 10 µg), gentamicin (GEN:10 µg), amikacin (30µg) ciprofloxacin (CIP: 5 µg), norfloxacin (NOR: 10 µg) and sulfamethoxazole-trimethoprim (SXT: 3.75/1.25 µg). The antibiotic disks were used from Abtek Biologicals Ltd, Liverpool, United Kingdom product.

### **Screening *Enterobacteriaceae* for ESBLs production**

Those *Enterobacteriaceae* which were resistant or reduced susceptibility to cefotaxime and/or ceftazidime (indicator cephalosporin(s)) were included as potential ESBL producers. In other word isolates that showed an inhibition zone size of ≤ 22 mm for ceftazidime (30 µg) and/or ≤ 27 mm for cefotaxime (30 µg) were considered as potential ESBL producers and selected for confirmation for ESBLs production [21].

### **Confirmation of ESBLs with combination disk test**

As per CLSI's recommendation, combination disk test (CDT) was performed with ceftazidime (30 µg) and cefotaxime (30 µg) disks with and without clavulanic acid (21). Moreover, disks containing 30µg cefepime and 30 µg cefepime/10 µg clavulanic acid were also prepared for this study as per EUCAST's recommendation [22]. The antibiotics were placed in appropriate distance on a MHA plate inoculated with a bacterial suspension of 0.5 McFarland turbidity standards and incubated overnight (16 – 18 hrs) at 37°C. An increase in the inhibition zone diameter of > 5 mm for a combination disk versus ceftazidime or cefotaxime or cefepime disk alone was considered as confirmed ESBLs producer.

### **Confirmation of ESBLs with double disk synergy test**

For the introduction of simple phenotypic methods for the detection and confirmation of ESBLs producing *Enterobacteriaceae*, the double disk synergy test (DDST) was tested and compared against CDT. The DDST was performed together with the AST according to the method developed by Jarlier and his colleagues and recommended by EUCAST [22,23]. Briefly, 0.5 McFarland suspension of the test isolate was swabbed on Muller Hinton Agar (MHA) plate and 30 µg antibiotic disks of ceftazidime, cefepime and cefotaxime were placed on the plate 20 mm (center to center) from the amoxicillin-clavulanate (20 µg/10 µg) disk and incubated at 37°C aerobically for 16 - 18 hrs.

Clear extension of the edge of the inhibition zone of cephalosporin toward the amoxicillin-clavulanate disk was interpreted as positive for ESBLs production.

**Quality control and data quality assurance**

ATCC 25922 *E. coli* standard strain was utilized as quality control to make sure that the culture media and antibiotics disks are work with in the expected performance. The quality of ESBLs confirmatory test was also checked using ESBLs positive *K. pneumoniae* ATCC 700603 and ESBLs negative *E. coli* ATCC 25922 control strains [21]. Data collection forms were checked to make sure that they are complete and accurate before data entry.

**Ethical considerations**

We got ethical clearance to conduct study from the departmental research and ethical review committee (DRERC) of the medical laboratory sciences, school of allied health sciences, College of Health Sciences; Addis Ababa University. A formal authorization was obtained from the laboratories in which the isolates were collected.

**Data entry and analysis**

Statistical software SPSS version 20 was used to enter and analyze data. The measure of agreement or kappa value between CDT and DDST was determined. Proportions and frequency of ESBLs producing *Enterobacteriaceae* isolate was used. The overall percent agreement, positive percent agreement (PPA) and negative percent agreement (NPA) were calculated.

**Results**

**Frequency of *Enterobacteriaceae***

The distribution of the 426 consecutive *Enterobacteriaceae* isolates is presented in table 1. The predominant isolates were *E. coli* with 53.5 % (228/426) and *K. pneumoniae* 24.1 % (103/426). In urine, *E. coli* were predominantly isolated bacteria with 82.5% (188/228), but they about 10.5% (24/228) from blood specimens. On other hand *K. pneumoniae* were obtained mostly from blood with 54.1% (53/103), followed by urine and wound/pus with 31.1% (32/103) and 11.6% (12/103) respectively.

Variables (Number)		<i>Enterobacteriaceae</i> isolate n (%)						
		<i>E. coli</i>	<i>K. pneumoniae</i>	<i>E. cloacae</i>	<i>Citrobacter. spp</i> s	<i>K. oxytoca</i>	<i>K. ozanae</i>	Other isolate
Bacteriology laboratories	ICL (150)	109 (72.7)	9 (6.0)	6 (4.0)	13 (8.7)	3 (2.0)	6 (4.0)	4 (2.6)
	EPHI (89)	36 (40.4)	29 (32.6)	6 (6.7)	6 (6.7)	2 (2.2)	4 (4.5)	6 (6.7)
	TASH (118)	53 (44.9)	33 (28.0)	7 (5.9)	9 (7.6)	9 (7.6)	1 (0.8)	6 (5.1)
	YHMC (69)	30 (43.5)	32 (46.4)	3 (4.3)	1 (1.4)	1 (1.4)	2 (2.9)	0 (0.0)
Types of Specimen	Urine (272)	188 (69.1)	32 (11.8)	11 (4.0)	19 (7.0)	7 (2.6)	7 (2.6)	8 (2.9)
	Blood (90)	24 (26.7)	53 (58.9)	3 (3.3)	2 (2.2)	5 (5.6)	3 (3.3)	0 (0.0)
	Pus (40)	8 (20.0)	12 (30)	6 (15.0)	4 (10.0)	2 (5.0)	3 (7.5)	5 (12.5)
	Sputum (6)	1 (16.7)	2 (33.3)	1 (16.7)	2 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)
	CSF (2)	0 (0.0)	2 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Body fluids (11)	5 (45.5)	2 (18.2)	1 (9.1)	1 (9.1)	1 (9.1)	0 (0.0)	0 (0.0)
	Ear and Eye discharge (5)	2 (40.0)	0 (0.0)	0 (0.0)	1 (20.0)	0 (0.0)	0 (0.0)	2 (40.0)
	Total (N = 426)	228 (53.5)	103 (24.1)	22 (5.2)	29 (6.8)	15 (3.5)	13 (3.1)	16 (3.8)

**Table 1:** Distribution of *Enterobacteriaceae* isolate against specimen types and bacteriology laboratory, Addis Ababa, Ethiopia.

Note: ICL: International Clinical Laboratories, EPHI: Ethiopian Public Health Institute, TASH: Tikur Anbessa Specialized Hospital, YMCH: Yekatit 12 Medical College Hospital

\*Other isolates are *P.mirabilis*, *Providencia spp*s, *M. morgani* and *E. aerogens*.



Screening and confirmation test result of *Enterobacteriaceae* isolates for ESBL production

The 265 isolates that were screening positive for ESBL were confirmed for ESBL production by CDT and DDST methods. The proportion of ESBL production using CDT and DDST was 57.7% 52.6% respectively. The majority of ESBL producers were found in *E. coli* (27.9%, 119/426), *K. pneumoniae* (19.0%, 81/426), and the other *Enterobacteriaceae* isolates accounted 10.8% (46/426). The highest intra-species frequency of ESBL production was observed among *K. pneumoniae* 78.6% (81/103) followed by *E. coli* and *Citrobacter species* with 52.2% (119/228) and 51.7% (15/29), respectively.

ESBL test result of double-disk synergy and combination disk test method

The isolates were screened for ESBLs production using indicator cephalosporin cefotaxime and ceftazidime antibiotics disks. Hence, cefotaxime identified 265 and ceftazidime 259 as potential ESBLs producing isolates. Of 265 *Enterobacteriaceae*, which were suspected for ESBLs production, 92.8% (246/265) were confirmed as ESBLs producer by CDT. The DDST, which was performed in conjunction with a routine disk diffusion test, detected ESBLs production in 224 out of 265 (84.5%) of the suspected isolates (Figure 1 and 2).

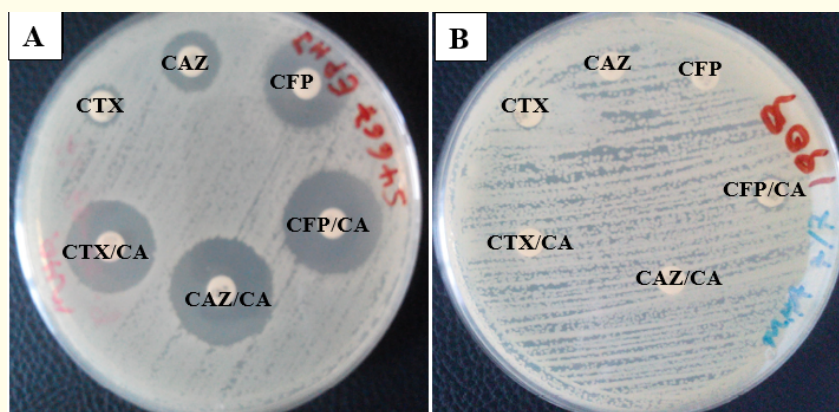


Figure 1: Combination Disk Test on 90mm plate: ESBLs positive (A) and negative (B) *Enterobacteriaceae*, Addis Ababa, Ethiopia.

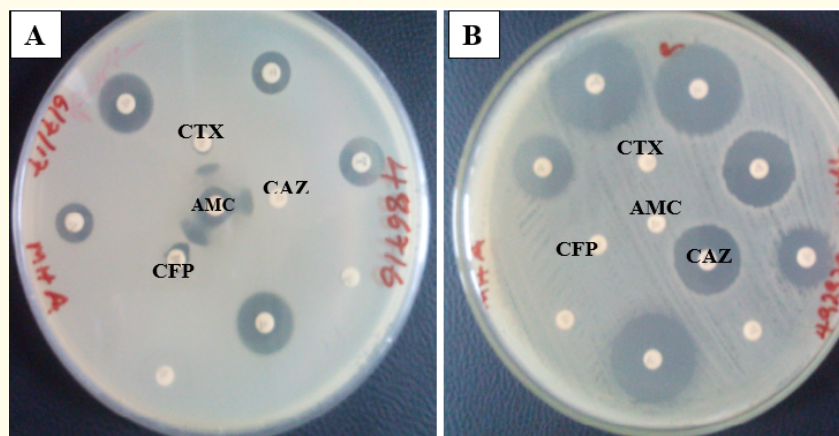


Figure 2: Double Disk Synergy Test on 150mm plate: ESBL positive (A) and ESBL negative (B) *Enterobacteriaceae*, Addis Ababa, Ethiopia.

Of 246 ESBLs positive *Enterobacteriaceae* by CDT, Double Disk Synergy Test (DDST) identified 224 (91.1%) of them by making synergy with one or/and two or three cephalosporins. Of 224 DDST ESBLs positive isolate, 13 (5.8%) isolate made synergy only with cefepime. The rest 207 isolates with the three disks and 4 isolates with cefotaxime and cefepime.

		Combination Disk Test method		Total
		Positive	Negative	
Double Disk	Positive	224	0	244
Synergy Test	Negative	22	19	41
Total		246	19	265

**Table 2:** Comparison of Double Disk Synergy Test and CLSI recommended Combination Disk test result Addis Ababa, Ethiopia.

From the table 1 we can calculate estimated overall percent agreement, positive percent agreement (PPA) and negative percent agreement (NPA).

$$\text{Overall percent agreement} = (a+d)/(a+b+c+d) \times 100\%$$

$$= (224+19)/(224+0+22+19) \times 100\%$$

$$=(243 / 265) \times 100\% = 91.7\%$$

$$\text{Positive percent agreement} = a/(a+c) \times 100\% = 224/(224+22) \times 100\% = 91.0\%$$

$$\text{Negative percent agreement} = d/(b+d) \times 100\% = 19/(0+19) \times 100\% = 100\%$$

The overall percent agreement between CDT and DDST was 91.7%, that was excellent (kappa value = 0.89; 95). The positive percent agreement (PPA) and negative percent agreement (NPA) between the two tests were also substantial, 91.0% and 100%, respectively.

**Antibiotics susceptibility pattern of ESBLs-E to potentially active drugs**

The highest antibiotics susceptibility proportion of ESBL positive *Enterobacteriaceae* to meropenem was 96.7%, followed by amikacin (82.1%) and cefoxitin (70%). Furthermore, the isolate were susceptible to gentamicin, ciprofloxacin and trimethoprim-sulfamethoxazole with 37%, 29% and 10.2% proportion, respectively. Non-ESBLs suspicious *Enterobacteriaceae* were 100%, 96.3% and 91.3% sensitive to meropenem, amikacin and cefoxitin respectively. Still, gentamicin and ciprofloxacin remained active against non-ESBLs suspicious *Enterobacteriaceae* with 90.1% and 70.2% sensitivity respectively.

**Discussions**

EESBLs producing *Enterobacteriaceae* are becoming one of the global public health burdens. Spread of ESBL producing bacteria compromise the activity of broad-spectrum antibiotics and affecting therapeutic success with a significant impact on patient outcome. This call for the utilization of simple, rapid, cost effective and reliable techniques for the identification of ESBL. The continued emergence of ESBLs presents diagnostic challenges to the clinical bacteriology laboratories [18].

In the present study, among the total 426 isolates, ESBLs producing *Enterobacteriaceae* was found to be 246 (57.7%) by CDT and 224 (52.6%) by DDST. These results were in good agreement with study done in India; Hooja S and his colleagues identified 60.2% by CDT and 56.8% by DDST [24] and Giriapur RS and his colleagues identified 63.89% by CDT and 56.23% by DDST as ESBLs producers [25]. This showed CDT was found to be better than DDST in the detection of ESBLs producing *Enterobacteriaceae*.

From ESBLs suspected isolate CDT identified 92.8% (246/265) of the isolates as ESBLs producers, whereas the DDST identified 84.5% (224/265) by making synergy with one and/or two or three cephalosporins. No *Enterobacteriaceae* isolate was found to be negative by CDT that was positive by DDST. Our finding was in agreement with a study conducted in Saudi Arabia: CDT (94%) and DDST (85%) [26], Ejaz H and his colleagues in Pakistan reported that CDT detected 99.5% while DDST detected 67.8% ESBLs producing isolate [27]. A study conducted in Ahmadabad also reported somewhat lower than our finding that CDT detected ESBLs in 85.4% (41/48) and DDST in 75% (36/48) of the isolates [28] and another study in Saudi Arabia by Kader, A A and his colleagues 112/136 (82%) and 102/136 (75%) were positive for ESBLs by the DDST and CDT methods, respectively [29].

In the present study, from ESBLs positivity by DDST, 5.8% (13/224) isolates showed synergy only with cefepime. Our finding was similar with study conducted in India by Kaur J and his colleagues reported 7.8% (17/218) [30], in Saudi Arabia by Kader and his colleagues found 10 (7.4 %) that showed a clear extension of the edge of inhibition by cefepime only [29]. If DDST were used without cefepime, 3.1% of ESBLs producing isolates from the total isolate could have been missed. The possible reason for missing may be the existence of chromosomally encoded or plasmid-encoded AmpC beta-lactamase enzyme that mask the synergistic effect of the clavulanic acid and the third generation cephalosporin against ESBLs and may thus lead to false negative ESBLs test results [31].

Different studies have reported that inappropriate initial antibiotic treatment for hospital acquired infection caused by ESBL-producing *Enterobacteriaceae* is associated with a significantly higher mortality rate than is initial therapy involving an antibiotics which is active against these ESBL-producing bacteria [32,33]. Therefore, prescribing drug after the laboratory detection of ESBL very necessary. In our study, although CDT had fairly better detection performance than DDST, the later might be the ideal test method where there is lack of combined antibiotics disks. In addition, instead of screening and confirming ESBLs production separately, one can perform directly the DDST along with routine antibiotic susceptibility testing. Performing ESBLs test routinely can help to report ESBLs production within 48 hours. The study showed that detection of ESBLs by DDST together with routine AST were essential for infection control and timely treatment of patient with best alternative antibiotics.

#### **Antibiotics susceptibility pattern of ESBLs producing *Enterobacteriaceae***

In this study, ESBLs producing isolates were found to be susceptible primarily to meropenem (96.7%), amikacin (82.1%) and ceftazidime (70%). This was in close agreement with study done in Ghana: meropenem (100%) [34], in Central India: meropenem (87.5%) and amikacin (83.92%) [35], in Jimma: amikacin (83.7%) [36], in India by Kaur, and his colleagues' meropenem (94.0%) and amikacin (82.6%) [30]. The results indicated that these antibiotics were the most active treatment of choice for ESBLs producing *Enterobacteriaceae*.

In this study, ESBLs producers showed higher resistance level against gentamicin (63%), trimethoprim-sulfamethoxazole (89.8%), ciprofloxacin (69%), cefepime (97.6%) and amoxicillin-clavulanic acid (91.5%). these results are comparable with the study conducted in Israel that showed 75% of ESBLs were non-susceptible to gentamicin, 70% to trimethoprim-sulfamethoxazole and 59% to ciprofloxacin [7], in Burkina Faso: 45% to trimethoprim-sulfamethoxazole, 89% to gentamicin, 80% to ciprofloxacin [37], in Ghana: 92.6% to trimethoprim-sulfamethoxazole, 91.2% to gentamicin and 41.1% to ciprofloxacin [34], in Central India: 50% to gentamicin, 87.5 % to ciprofloxacin and 94.6% to trimethoprim-sulfamethoxazole [35], and in Nepal: 90.7% to ciprofloxacin, 90.4% to trimethoprim-sulfamethoxazole, 63.12% to gentamicin [38]. Our findings indicated that these ESBLs production among nosocomial *Enterobacteriaceae* is also the main reason for the development resistance to different antibiotics families.

#### **Limitation of the Study**

We didn't use molecular method as a reference standard to evaluate the performance of the two test methods, although sensitivity and specificity of test methods are usually determined against a reference standard test, or 'gold standard' test. Indeed, if a reference standard is not available, an appropriate approach might be used to report the levels of agreement between different tests, that is, positive or negative by both test method [39].

## Conclusion and Recommendation

The performance agreement of DDST and CLSI recommended CDT was found to be very good. Early detection of ESBLs producing *Enterobacteriaceae* in clinical laboratories is essential for the management of patients infected with ESBLs producing *Enterobacteriaceae*. If DDST is performed in conjunction with the routine antibiotics susceptibility, it will help clinicians in selecting and prescribing proper antibiotics for treatment of infections caused by ESBLs producing and for the control of the dissemination of such bacteria. If a laboratory detect ESBLs producing bacteria patient's report must state that the isolate is a suspected or confirmed ESBLs producer and also include a note stating that ESBLs production may predict therapeutic failure with penicillins, all cephalosporins (except cephamycins) and aztreonam, irrespective of their *in vitro* susceptibility.

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## Conflict of Interest

The authors declare that they have no conflict of interest.

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