Journal of High Institute of Public Health 2020;50(3):132-138.

Original Article

Evaluation of Nordmann–Dortet–Poirel Test as a Rapid and Direct Method of Detection of Extended-Spectrum β-Lactamases in Urinary Tract Infections

Laila A. El-Attar [1], Manal M. Baddour [2], Heba H. Omar [3 ¥], Marwa M. Fekry [1]

[1] Department of Microbiology, High Institute of Public Health, Alexandria University, Egypt

[2] Department of Microbiology and Immunology, Faculty of Medicine, Alexandria University, Egypt

[3] Clinical Pharmacy, Alexandria Main University Hospital, Alexandria University, Egypt

Abstract

Background & Objective(s): Antimicrobial resistance due to extended-spectrum β-lactamase (ESBL) production is a major public health issue. Its rapid detection is critical for early appropriate antibiotic use to prevent treatment failure, especially in cases of septicemia requiring appropriate empiric antibiotic therapy within the first few hours, thereby decreasing the mortality rate. Rapid detection is also important to spare the use of carbapenems, which, if used as a first-line drug in antibiotic policies, may lead to the emergence and spread of carbapenemases.

We evaluated the Nordmann–Dortet–Poirel (NDP) test as a rapid method to detect ESBL producers directly from urine samples from patients with symptomatic urinary tract infections (SUTIs). Furthermore, we determined the clinical and economic outcomes of using NDP test results to guide antibiotic therapy.

Methods: This cross-sectional study and double-blind, randomized control trial was conducted over 10 months. Urine samples were collected randomly from all patients with urinary tract infections admitted to the Internal Medicine Department at Alexandria University Hospital during the study period and assessed for eligibility. We enrolled 152 SUTI patients with gram-negative bacilli ($\geq 10^5$ cfu/ml), and the samples were tested for ESBLs using modified double-disk synergy testing (MDDST) and the NDP test. Patients were randomly divided into groups A or B, where culture-based therapy or NDP test-guided therapy was used first, respectively. All patients were observed for a clinical cure for at least 5 days.

Results: The prevalence of ESBLs was 50% using MDDST. The overall sensitivity, specificity, positive predictive value, negative predictive value, and total accuracy for the ESBL NDP test performed directly on urine samples, using interpretable results, were 89.86%, 62.86%, 70.45%, 86.27%, and 76.26%, respectively. There was moderate agreement between the NDP test and MDDST and a statistically significant reduction in the length of antibiotic therapy (LOT) in the group using NDP test-guided therapy ($p = 0.0002$).

Conclusion: The NDP test is a rapid and easy ESBL detection method that could be introduced in clinical practice. It is useful in guiding empiric therapy and reducing the LOT. A combination of ESBL NDP and Carba NP tests could be used in areas with a high prevalence of carbapenemases and ESBLs, but further studies are necessary to confirm efficacy.

Keywords: Nordmann–Dortet–Poirel test, modified double-disk synergy test, extended-spectrum β-lactamase detection, urinary tract infection

Available on line at:
www.jhiph.alexu.edu.eg

Print ISSN: 2357-0601
Online ISSN: 2357-061X

¥*Correspondence*:
Email: heba.hani@alexmed.edu.eg

Suggested Citations: El-Attar LA, Baddour MM, Omar HH, Fekry MM. Evaluation of Nordmann–Dortet–Poirel Test as a Rapid and Direct Method of Detection of Extended-Spectrum β-Lactamases in Urinary Tract Infections. JHIPH. 2020;50(3):132-138.

INTRODUCTION

Antibiotic use in Egypt has greatly increased from 2000 to 2015, including a dramatic increase in the defined daily dose of cephalosporins and quinolones per 1000 population.(1) The irrational use of antibiotics, especially third-generation cephalosporins (3GCs), has resulted in the spread of antibiotic resistance among gram-negative bacteria, which is a major concern.(2) One of the resistance mechanisms is the production of extended-spectrum β-lactamases (ESBLs). These enzymes hydrolyze extended-spectrum cephalosporins and are

inhibited by β-lactamase inhibitors, but they cannot hydrolyze carbapenems efficiently.[3]

The prevalence of ESBLs in Egypt is high and was reported as 36%–88.6% in urinary tract infections (UTIs) in different areas of Egypt.[4,5] ESBL production results in resistance to extended-spectrum cephalosporins, which are used as empiric therapy for many infections according to international guidelines, leading to delays in receiving effective antibiotic therapy, increased lengths of hospital stays, increased overall healthcare costs, and higher mortality.[6,7] Thus, their rapid detection is crucial because in addition to the prevention of treatment failure, it is also important in sparing the use of carbapenems, which, if used as first-line treatment as hospital policy in settings of high ESBL prevalence, may lead to the emergence of carbapenemases.[8]

Phenotypic detection of ESBL producers is based on the identification of susceptibility to expanded-spectrum cephalosporins (screening) followed by the inhibition of the ESBL activity with the use of clavulanate or tazobactam (confirmatory test). One of these confirmatory tests is the double-disk synergy test, which has the disadvantage of requiring up to 48 h for ESBL detection.[9] Polymerase chain reaction assay is an alternative molecular detection method but is costly, requires bacterial isolation, and fails to detect all genes encoding ESBLs. Therefore, a rapid technique is required.[10]

The Nordmann–Dortet–Poirel (NDP) test was developed based on the identification of the hydrolysis of the β-lactam ring of cefotaxime, generating a carboxyl group. The acidity resulting from this hydrolysis is identified by the yellow color produced using a pH indicator (phenol red). Inhibition of ESBL activity is performed with the addition of tazobactam. The results of this test can be obtained within 30 min.[11].

This cross-sectional study and double-blind, randomized control trial evaluates the NDP test for rapid ESBL detection in UTIs and its effect in guiding therapy.

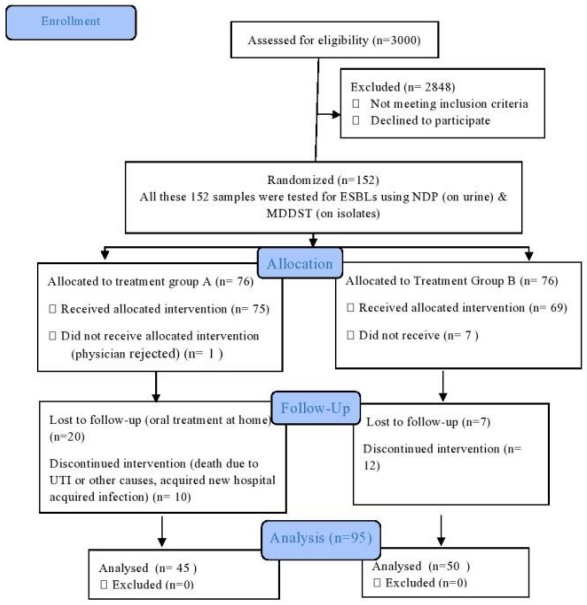
METHODS

Sample size

The sample size was calculated based on the findings of Dortet et al. (2014), who reported that 11.3% of strains in urine samples were positive for ESBL.[12] It was estimated that 4000 patients per year were eligible for testing. Thus, 149 specimens were considered sufficient for the sample size to be statistically significant, with 80% power and at a significance level of 0.05.[13,14]

Patients and samples

This cross-sectional study and double-blind, randomized control trial was conducted over 10 months from June 2018 to April 2019. Clean-catch midstream urine samples were collected aseptically from 3000 patients with UTIs admitted to the Internal Medicine Department at Alexandria University Hospital during the study period and assessed for eligibility. Pregnant females and patients with asymptomatic UTIs, hypersensitivity to meropenem, a history of seizures, loss of consciousness at the time of randomization, and anuria were excluded. Finally, 152 urine samples of patients who fulfilled the inclusion criteria of having symptomatic urinary tract infection (SUTI) with gram-negative bacilli (GNB; $\geq 10^5$ cfu/ml) were selected, and the patients were enrolled in our study and the randomized controlled trial (Figure 1).



Procedure

We collected 3000 urine samples from patients with SUTIs and stored the samples for 24 h in a refrigerator. Each sample was cultured on blood agar (semiquantitative culture) and MacConkey agar (selective medium) for bacterial isolation. Of them, 152 samples showed GNB $\geq 10^5$ cfu/ml, which fulfilled the inclusion criteria, and these patients were enrolled in our study. The isolates were identified according to standard microbiological methods[15] by antibiotic susceptibility testing and ESBL screening using the disc diffusion method and underwent ESBL confirmatory tests using modified double-disk synergy testing (MDDST). Furthermore, all 152 urine samples were tested for ESBLs using the NDP test. The NDP test and MDDST were performed simultaneously on the isolates.

Antibiotic susceptibility testing (disk diffusion method) and ESBL detection (MDDST) of bacterial

Mueller–Hinton agar plates were streaked with cotton swabs immersed in the bacterial suspension adjusted to 0.5 McFarland standard. These plates were used to determine the susceptibility of the isolates to antimicrobials and to detect ESBLs using MDDST. The discs of a 3GC (30 μg cefotaxime) and fourth-generation cephalosporin (4GC) (30 μg cefepime) were placed 20 mm apart center-to-center to that of an amoxicillin-clavulanate disc and angled at 180° apart from each another. The inhibition zones were measured, and susceptibility was recorded according to the

standard tables of Clinical and Laboratory Standards Institute (CLSI) following incubation for 24 h at 37 °C. Any increase in the zone toward the amoxicillin-clavulanate disk with either 3GC or 4GC was considered indicative of ESBL producers, while clavulanate synergy with only 4GC (cefepime) and not 3GCs was considered indicative of ESBL and AmpC co-producers.[16,17]

ESBL NDP test using urine samples

Each urine sample was dispensed into three marked Eppendorf tubes (A, B, and C; 1.5 ml each). These were centrifuged at 8,765 x g for 3 min, and the supernatant was discarded. The pellet in each tube was resuspended in 500 µl of dH_2O. Centrifugation was repeated, and the supernatant was discarded. The pellet in each tube was resuspended in 100 µl of B-PER Bacterial Protein Extraction Reagent Thermo Fisher Scientific for cell lysis to obtain an enzymatic suspension. To tube A, 100 µl of the revelation solution R (prepared by mixing 2 ml of a vortexed 0.5% phenol red suspension in 16.6 ml of dH_2O and then adjusting the pH to 7.8 by adding drops of 1 N NaOH) was added. To tube B, 100 µl of cefotaxime solution (6 mg/ml cefotaxime powder in solution R prepared in situ) was added. To tube C, 10 µl of tazobactam solution (40 mg/ml) was added first, followed by 100 µl of cefotaxime solution. The three tubes were incubated at 37 °C for a maximum of 30 min and observed for color change by the naked eye (Table 1). Solution R and tazobactam solution were stored at −20 °C.[12]

Table 1: Interpretation of NDP test results

Results			Interpretation
No antibiotic (tube A)	Cefotaxime (tube B)	Cefotaxime + tazobactam (tube C)	
Red	Yellow	Red	ESBL (positive)
Red	Red	Red	Non-ESBL (negative)
Red	Yellow	Yellow	†Cephalosporinase or cephalosporinase + ESBL
Yellow	Yellow	Yellow	Uninterpretable

†Considered negative

Randomization

The 152 patients enrolled in this 1:1 concealed allocation, parallel double-blind (physician, patient, and statistician) randomized control trial were divided into two study groups (A or B) according to a computer-based randomization schedule using the permuted block technique with variable block size.

Most of the study population was empirically treated by the responsible physician according to international guidelines. Study group A started with 76 patients, and the antibiotic regimen was initiated or changed according to the culture results only. Forty-five patients completed the therapy. The other patients were lost due to death or follow-up failure, which was due to early hospital discharge (either at the demand of the patient or due to the prescription of an oral antibiotic at home) or due to the new acquisition of a hospital-acquired infection during therapy. Study group B started with 76 patients who were treated according to their response to empiric therapy as follows:

1. For those who were responding, the antibiotic regimen remained unchanged regardless of the NDP test results.
2. For those who did not start empiric therapy and those who were not responding, the antibiotic regimen was either initiated or changed according to the NDP test results.
3. For those who showed a positive NDP test result, the regimen was initiated or changed to carbapenem or fosfomycin.
4. For those with a negative NDP test result, the regimen was initiated or changed according to the culture results.

Only 50 patients in group B completed the therapy. The other patients were lost due to death or follow-up failure during therapy.

All 95 enrolled patients who completed treatment were observed for at least 5 days based on a clinical cure (complete resolution of UTI symptoms or urosepsis symptoms, as evaluated by a blinded investigator). We recorded and compared the number of deaths during the follow-up period, number of deaths due to UTI, length of antibiotic therapy (LOT), and the total cost for both groups (secondary outcomes).

Ethical considerations

Ethical Committee approval was obtained from the High Institute of Public Health and institutional approval was obtained from Alexandria University Hospital prior to study commencement. Informed consent to participate and for publication was obtained from all participants prior to study enrolment. All procedures were performed in accordance with the principles Declaration of Helsinki.

Statistical analysis

Data were collected and captured using WHONET 2018 version 8.6 microbiology laboratory database software downloaded from https://www.whonet.org/, SPSS v21 for statistical analysis from Arab Academy for Science and Technology, and Epi Info v5 software downloaded from https://www.cdc.gov/epiinfo/. The bacterial susceptibility data were interpreted using CLSI 2018 breakpoints for each antibiotic.(16) Chi-square tests were used to test the association between qualitative variables, and paired t-tests were used to test the association between continuous variables.

RESULTS

The demographic data and clinical characteristics of the patients in both study groups are presented in Table 2.

Among the 152 GNB ≥105 cfu/ml isolated from urine samples from patients with SUTIs, Escherichia coli was the most commonly isolated organism, whether a community-acquired UTI (com-UTI; 72/116; 62.07%) or a hospital-acquired UTI (HAUTI; 15/36; 41.67%), followed by Klebsiella pneumoniae with 25.0% (29/116) and 36.11% (13/36), respectively (Table 3).

According to susceptibility testing results of the 152 GNB $\geq 10^5$ cfu/ml, 34.21% were considered carbapenemase producers and were resistant or intermediate to either imipenem or meropenem or both.

The prevalence of ESBL producers in urine was found to be 50% using MDDST (Table 2). It was slightly higher among males (38/67; 56.72%) than among females (38/85; 44.71%) and in com-UTIs (61/116; 52.59%) compared with HAUTIs (15/36; 41.67%), but this was not statistically significant. (p = 0.14147 and 0.2523, respectively).

According to the susceptibility testing results (Table 4.B), the most effective antibiotics to treat UTIs caused by ESBL producers were colistin and fosfomycin because none of the isolates showed resistance toward them. These were followed by amikacin (1.41% resistance), meropenem (5.33% resistance), and imipenem (5.48% resistance). On the other hand, all ESBL producers were resistant to ceftriaxone, followed by cefotaxime (98.67% resistance). The NDP test result was obtained 30 min from urine collection, whereas culture and MDDST had a turnaround time of at least 48 h. The overall sensitivity, specificity, positive predictive value, and negative predictive value for the identification of ESBLs using the NDP test with interpretable results directly on urine samples was 89.86%, 62.86%, 70.45%, and 86.27%, respectively. There was moderate agreement between the NDP test and MDDST for ESBL detection (Table 5). A statistically significant reduction in the LOT was found in group B using NDP test-guided antibiotic therapy. However, the mortality rate due to UTIs was not significantly higher in group A (4.44%) compared with group B (Table 6).

Table 2: Demographic data and clinical characteristics of both study groups

Characteristic	Cross-sectional study (n = 152)		Randomized controlled trial			
			Group A (n = 45)		Group B (n = 50)	
	n	%	n	%	n	%
Sex						
Female	85	55.92	27	60.00	23	46.00
Male	67	44.08	18	40.00	27	54.00
Age						
Adults (18–64 years)	100	65.79	29	64.44	34	68.00
Older adults (>65 years)	52	34.21	16	35.56	16	32.00
UTI acquisition setting						
Com-UTI	116	76.32	35	77.78	41	82.00
HAUTI	36	23.68	10	22.22	9	18.00
Type of UTI						
Cystitis			12	26.67	11	22.00
Pyelonephritis			33	73.33	39	78.00
ESBL identification by MDDST						
Positive	76	50.00	28	62.22	23	46.00
Negative	76	50.00	17	37.78	27	54.00
Total	152	100.00	45	100.00	50	100.00

UTI = urinary tract infection Com-UTI = community-acquired urinary tract infection HAUTI = hospital-acquired urinary tract infection ESBL = extended-spectrum β-lactamase MDDST = modified double-disk synergy testing

Table 3: Distribution of the 152 isolated GNB (≥ 105 cfu/ml) from the urine samples of 152 patients with SUTIs, according to their setting of acquisition, Alexandria, 2018–2019

Isolates	Com-UTI			HAUTI			Total		$\chi^{2§}$	p-value
	n	%$_1$†	%$_2$‡	n	%$_1$	%$_2$	n	%		
Escherichia coli	72	82.76	62.07	15	17.24	41.67	87	57.24	4.672	0.031*
Klebsiella pneumoniae	29	69.05	25.00	13	30.95	36.11	42	27.63	1.696	0.193
Pseudomonas aeruginosa	5	50.00	4.31	5	50.00	13.89	10	6.58	2.691(Y)¶	0.101
Acinetobacter baumannii	4	66.67	3.45	2	33.33	5.56	6	3.95	0.006(Y)	0.9383
Enterobacter cloacae	2	100.00	1.72	0	00.00	0.00	2	1.32	0.000(Y)	1.000
Burkholderia cepacia	1	50.00	0.86	1	50.00	2.78	2	1.32	0.002(Y)	0.965
Morganella morganii	1	100.00	0.86	0	00.00	0.00	1	0.66	0.000(Y)	1.000
Proteus mirabilis	1	100.00	0.86	0	00.00	0.00	1	0.66	0.000(Y)	1.000
Citrobacter freundii	1	100.00	0.86	0	00.00	0.00	1	0.66	0.000(Y)	1.000
Total	116	76.32	100.00	36	23.68	100.00	152	100.00		

*p-values <0.05 were considered statistically significant †%1 = row percentage ‡%2 = column percentage §Chi-square test¶Yates' corrected Chi-square test Com-UTI = community-acquired urinary tract infection HAUTI = hospital-acquired urinary tract infection

Table 4.A: Collective antimicrobial resistance pattern of the 152 isolated GNB (≥ 105 cfu/ml) in patients with Com-UTI and HAUTI, Alexandria, 2018–2019

Antibiotics	Com-UTI	HAUTI	$\chi^{2\dagger}$	*p*-value
	Resistance (R) %			
AMK	23.85	35.29	1.739	0.1873
GEN	51.38	47.06	0.193	0.6602
AMC	53.64	80.00	7.689	0.006*
SAM	76.74	81.25	0 (Y)§	0.99
CSL	35.29	55.56	3.666	0.0555
TZP	36.04	52.94	3.099	0.078
FEP	73.45	76.47	0.124	0.724
CTX	81.08	91.43	2.074	0.150
CAZ	71.82	74.29	0.081	0.776
CRO	82.73	90.00	0.47 (Y)	0.49
CIP	81.55	77.42	0.260	0.610
LVX	81.31	75.00	0.609	0.435
NOR	83.05	76.47	0.07 (Y)	0.79
COL	30.77	8.33	1.963	$^{FE}p = 0.322$¶
FOS	4.67	3.33	0 (Y)	1
IPM	22.02	45.45	6.984	0.0082*
MEM	23.68	41.67	4.389	0.0362*
NIT	25.93	42.86	3.070	0.080
SXT	70.00	75.00	0.01(Y)	0.93

**p*-values <0.05 were considered statistically significant †Chi-square test ‡number of tested GNB §Yates' corrected Chi-square
¶ Fisher's exact test Com-UTI = community-acquired urinary tract infection HAUTI = hospital-acquired urinary tract infection

Table 4.B: Collective antimicrobial resistance pattern of the 76 ESBL producers, Alexandria 2018-2019

Antibiotic	ESBLs
	Resistance %
AMK	1.41
GEN	42.47
AMC	44.00
SAM	80.00
CSL	18.31
TZP	22.97
FEP	87.84
CTX	98.67
CAZ	84.93
CRO	100.00
CIP	85.71
LVX	89.71
NOR	84.62
COL	0.00
FOS	0.00
IPM	5.48
MEM	5.33
NIT	15.07
SXT	68.29

AMK = amikacin GEN = gentamicin
AMC = amoxicillin-clavulanate
SAM = ampicillin/sulbactam
CSL = cefoperazone/ sulbactam
TZP = piperacillin/tazobactam
FEP = cefepime CTX = cefotaxime
CAZ = ceftazidime CRO = ceftriaxone
CIP = ciprofloxacin LVX= levofloxacin
NOR= norfloxacin COL = colistin FOS = fosfomycin
IPM = imipenem MEM = meropenem
NIT= nitrofurantoin
SXT = sulfamethoxazole/trimethoprim

Table 5: Agreement between NDP test and MDDST (GNB ≥105 cfu/ml) for ESBL detection among the 152 urine samples from patients with SUTIs patients, Alexandria, 2018–2019

NDP test results within 30 min		MDDST results after 48 h					
		Negative		Positive		Total	
		n	%	n	%	n	%
Interpretable	Negative	44	31.65	7	5.04	51	36.69
	Positive	26	18.71	62	44.60	88	63.31
	Sub-Total	70	50.36	69	49.64	139	100.0
Non-interpretable		6	46.15	7	53.85	13	100.0
Total		76	50.00	76	50.00	152	100.0
Calculation performed for total GNB using interpretable result	Kappa	0.526					
	Sensitivity	89.86%					
	Specificity	62.86%					
	Positive predictive value	70.45%					
	Negative predictive value	86.27%					
	Total accuracy	76.26%					

NDP, Nordmann–Dortet–Poirel MDDST = modified double-disk synergy testing GNB = gram-negative bacilli

Table 6: Effect of using NDP test-guided antibiotic therapy on total cost and LOT

		Using the number of patients					
		Group A (n = 45)		Group B (n = 50)		$\chi^{2\dagger}$	*p*-value
		n	%	n	%		
Total cost	Decreased	3	6.67	17	34.00	10.65	0.0011*
	Increased	16	35.56	5‡	10.00	8.983	0.0027*
	Equal	26	57.78	28	56.00	0.03051	0.8619
LOT	Decreased	1	2.22	16	32.00	14.29	0.0002*
	Increased	16	35.56	0	0.00	21.38	0.000004*
	Equal	28	62.22	34	68.00	0.3488	0.5548
Mortality due to UTI		2	4.44	1	2.00	0.0086(Y)	0.926

Using the mean LOT and mean cost (continuous variables)				
	Mean	Group A	Group B	$t^{\ddagger}$	Significance level (two-tailed)
LOT (days)		9.653	9.000	6.245	0.000*
Cost of LOT (LE)		579.160	540.000	6.245	0.000
Cost of treatment (LE)		1513.1552	1510.7656	0.084	0.933
Total cost (LE)		2092.3100	2050.7656	1.393	0.167

*p-values < 0.05 were considered statistically significant † Chi-square test ‡Paired sample t-test
LOT = length of antibiotic therapy UTI = urinary tract infection LE = Egyptian pounds

DISCUSSION

We found that 50% of the isolates in our study were ESBL producers using MDDST, which was similar to the results recorded at the Theodor Bilharz Research Institute, Giza, Egypt, in 2016 (49%).[18] The high ESBL prevalence in our study and in others in Egypt might be due to the irrational high use of antibiotics, especially 3GCs.[2]

In 2012 at Bicêtre University Hospital, France, and in 2016 at Benha University, Egypt, NDP tests performed directly on urine samples showed a sensitivity of 90.5%–98% and specificity of 99.8%–100%,[12,19] which was higher than the results obtained using the NDP test in our study. The low specificity (62.86%) in our study was due to the high number of false-positive NDP test results, which represented 17.11% (26/152) of the total results. Of them, ~20% could not be explained, whereas ~80% were carbapenem non-susceptible (CNS) isolates. These CNS isolates might be Ambler class A carbapenemase producers (KPC producers), which hydrolyze both carbapenems and cefotaxime, with their hydrolytic activity being inhibited by tazobactam, thereby giving positive NDP results.[11] The lower sensitivity of 89.86% in our study was due to false-negative NDP test results, which represented 4.61% (7/152) of the total results. These false-negative results might be due to the lack of detection of TEM- or SHV-type ESBL producers that have weak cefotaxime hydrolytic activity, leading to minimal color change, or due to the presence of AmpC co-producers (by MDDST), which represented 42.86% of the false-negative results. These cephalosporinases hydrolyze cefotaxime without being inhibited by tazobactam, leading to a color change in both the tube with cefotaxime and the tube with the tazobactam in addition to cefotaxime. False-negative results might also be due to the presence of CNS isolates, which represented 42.86% of the false-negative results. These CNS isolates might be co-producers of Ambler class B carbapenemases of VIM-, IMP-, and NDM-types that hydrolyze both carbapenems and cefotaxime but are not inhibited by tazobactam, leading to a color change in both the tube with cefotaxime and the tube with tazobactam in addition to cefotaxime, or co-producers of Ambler class D carbapenemases of OXA-48 type that hydrolyze carbapenems but do not hydrolyze cefotaxime. These do not result in color change in any tube.[11,12]

In our study, the cost of the reagents for the ESBL NDP test per sample using Alfa Aeser tazobactam sodium (Thermo Fisher Scientific) was approximately 18.34 Egyptian pounds (LE)/sample, with the most expensive reagent being B-PER (11.06 LE), accounting for approximately 60.31% of the total cost. This was different from a study by Affolabi et al., where the cost per sample was US$ 7.3 (115.56 LE), with the most expensive reagent being the tazobactam salt (US$ 6.6, ~104.48 LE), which represented 90.4% of the total cost. It is supposed that if the NDP test is implemented in routine practice, it might require additional costs, including personnel and laboratory costs, such as electricity, Eppendorf tubes, pipettes, and gloves.[20]

There was a statistically significant reduction in LOT for patients who were given NDP test-guided antibiotic therapy in our randomized control trial. This might be due to the delay in the appropriate antibiotic intake in the group using standard culture-based therapy.

The difference in the mean cost of antibiotic treatment was not significantly lower in patients using NDP test-guided therapy. This might be because carbapenems were used to treat six patients infected by CNS isolates that were falsely considered as ESBL-positive by the NDP test.

The limitation of our study was the absence of ESBL and carbapenemase molecular detection tests for each sample to confirm the interpretation of the NDP test

results. In areas with a high prevalence of carbapenemases and ESBLs, a combination of ESBL NDP and Carba NP tests is suggested, but further studies are necessary to confirm its efficacy.

CONCLUSION AND RECOMMENDATIONS

The NDP test is a rapid and easy ESBL detection method that could be introduced in clinical practice. It is useful in guiding first-line empiric therapy and reducing LOT. In areas of a high prevalence of carbapenemases and ESBLs, a combination of ESBL NDP and Carba NP tests is suggested, but further studies are required to confirm its efficacy.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

FUNDING

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CODE AVAILABILITY: Each patient enrolled was coded for statistical analysis. The code is available in Mendeley at https://data.mendeley.com/datasets/9f5yprwr7g/drafta=32589c4a-b691-4554-a81d-966026a14ae6

AVAILABILITY OF DATA AND MATERIALS: Data generated or analyzed during the study are included in this published article. Data supporting our study findings are available in the Mendeley data repository at https://data.mendeley.com/datasets/9f5yprwr7g/draft?a=32589c4a-b691-4554-a81d-966026a14ae6.

REFERENCES

1. Center for Disease Dynamics, Economics & Policy. Resistance map: Antibiotic use in Egypt. 2020. Available online at https://resistancemap.cddep.org/countrypage.php?countryId=74&counry=Egypt. Accessed [10 March 2020].
2. Adesoji A, Onuh JP, Okunye OL. Bacteria resistance to cephalosporins and its implication to public health. J Bacteriol Mycol. 2016;3(1):1-6.
3. Shaikh S, Fatima J, Shakil S, Rizvi SMD, Kamal MA. Antibiotic resistance and extended-spectrum β-lactamases: types, epidemiology and treatment. Saudi J Biol Sci. 2015;22(1):90-101.
4. Elsayed TI, Ismail HAF, Elgamal SA, Gad HA. The occurrence of multidrug resistant E. coli which produce ESBL and cause urinary tract infections. J Appl Microbiol Biochem. 2017;1(2):8.
5. Fattouh M, Goda AM, Bakry MM, Abo Zaid AM. Prevalence and molecular characterization of extended-spectrum β-lactamases producing Escherichia coli isolates causing hospital-acquired and community-acquired urinary tract infections in Sohag University Hospitals, Egypt. Egypt J Med Microbiol. 2017;26(1):49-59.
6. Centers for Disease Control and Prevention. Antibiotic resistance threats report. 2019. Available online at www.CDC.gov/DrugResistance/Biggest-Threats.htm/. Accessed [14 March 2020].
7. Wilson H, Estée Török M. Extended-spectrum β-lactamase producing and carbapenemase producing Enterobacteriaceae. Microb Genom. 2018;4(7):e000197.
8. Viale P, Giannella M, Bartoletti M, Tedeschi S, Lewis R. Considerations about antimicrobial stewardship in settings with epidemic extended-spectrum β-lactamase-producing or carbapenem-resistant Enterobacteriaceae. Infect Dis Ther. 2015;4(1):65-83.
9. Rawat D, Nair D. Extended-spectrum β-lactamases in gram negative bacteria. J Glob Infect Dis. 2010;2(3):263-74.
10. Gazin M, Paasch F, Goossens H, Malhotra-Kumar S. Current trends in culture-based and molecular detection of extended-spectrum β-lactamase harboring and carbapenem resistant Enterobacteriaceae. J Clin Microbiol. 2012;50(4):1140-46.
11. Nordmann P, Dortet L, Poirel L. Rapid detection of extended-spectrum-β-lactamase-producing Enterobacteriaceae. J Clin Microbiol. 2012;50(9):3016-22.
12. Dortet L, Poirel L, Nordmann P. Rapid detection of extended-spectrum-β-lactamase producing Enterobacteriaceae from urine samples by use of the ESBL NDP test. J Clin Microbiol. 2014;52(10):3701-6.
13. Daniel W. Biostatistics. A Foundation for Analysis in the Health Sciences. 6th ed. New York: Wiley & Sons; 1995.
14. Killeen P. An alternative to null-hypothesis significance tests. Psychol Sci. 2005;16(5):345-53.
15. Tille PM. Bailey & Scott's Diagnostic Microbiology. 13th ed. St. Louis, Missouri: Mosby Inc.; 2014.
16. Clinical and Laboratory Standards Institute. M100 Performance Standards for Antimicrobial Susceptibility Testing. 28th ed. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.
17. Dhara M, Disha P, Sachin P, Manisha J, Seema B, Vegad MM. Comparison of various methods for the detection of extended-spectrum β-lactamase in Klebsiella pneumoniae isolated from neonatal intensive care unit. National J Med Res. 2012;2(3):348-53.
18. Zaiton M, Din S, Hamid N, Salem D, Sheikh S. Rapid detection of extended-spectrum β-lactamases (ESBL) and their CTX-M genetic characterization. Int J Adv Res. 2018;6(7):60-8.
19. Saeed AM, Tabl HA, Mogahed MM. Evaluation of the NDP test, a novel chromogenic test for rapid detection of extended-spectrum β-lactamase producing Enterobacteriaceae. Microbiol Res J Int. 2017;18(6):1-8.
20. Affolabi D, Sogbo F, Laleye G, Orekan J, Massou F, Kehinde A, et al. Rapid detection of extended-spectrum-β-lactamase-producing Enterobacteriaceae in blood cultures using ESBL NDP test in Cotonou, Benin. J Med Microbiol. 2017;66:884-7.