

RESEARCH ARTICLE

Screening of Extended-Spectrum β -Lactamases (ESBL)-producing *Klebsiella pneumoniae* with ChromID ESBL Media

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Received date: Jan 23, 2018; Revised date: May 30, 2018; Accepted date: Jun 5, 2018

Abstract

BACKGROUND: *Klebsiella pneumoniae*, one of clinical isolates, is frequently found causative agent of hospital acquired infection. Currently, *K. pneumoniae* is found as extended-Spectrum β -lactamases (ESBL) producer, allowing it to become multidrug-resistant. A clinical laboratory with limited facility needs a valid, reliable, inexpensive and simple laboratory test to control its infection and antimicrobial-resistance. The aim of this study is to evaluate the diagnostic performance of a ESBL media to detect ESBL-producing *K. pneumoniae*.

METHODS: An independent and blind comparative study of ChromID ESBL media and Double Disc Synergy Test (DDST) was conducted for detecting the clinical isolate of ESBL-producing *K. pneumoniae*. Clinical isolates of *K. pneumoniae* collected from the Clinical Laboratory of Dr. Sardjito Hospital were isolated.

RESULTS: There were 103 clinical isolates of *K. pneumoniae*, which were isolated from urine, pus, blood, stool, cerebrospinal fluid, sputum, drain liquid, nasal sinus liquid, gastric wash, bronchi liquid, injury liquid and nasal swab. The number of true positive, true negative, false positive and false negative results were 74, 18, 9 and 2, respectively. Meanwhile, the sensitivity, specificity, positive predictive value, negative predictive value, likelihood ratio for positive result and likelihood ratio for negative result of the new ESBL media were 97.4%, 66.7%, 89.2%, 90%, 2.9 and 0.03, respectively.

CONCLUSION: Since the new ESBL media and DDST results were similar, so the new ESBL media could be used for screening patients with clinical presentation that indicating a high suspicious of ESBL-producing bacteria.

KEYWORDS: *K. pneumoniae*, ChromID ESBL, DDST, ESBL, sensitivity

Indones Biomed J. 2018; 10(3): 217-21

Introduction

In developing countries, infectious diseases remain as the first cause of illness in community. Treatments for infections usually involve antibiotics. With many advances in technology, the number and type of clinically useful antibiotics is increasing. Inappropriate antibiotic treatment may result adverse effects, such as the emergence of bacterial resistance and low antibiotic efficacy against

certain bacteria. Bacterial resistance against antibiotics are a current global issue.(1)

Resistance against antibiotics is mostly caused by nosocomial infections. *Klebsiella spp.* are important pathogenic bacteria causing nosocomial infections. Generally, *Klebsiella spp.* infections are implicated in pneumonia, urinary tractus infections, meningitis and septicemia. The increasing resistancy of *Klebsiella spp.* has been widely reported as the effect of enzymes extended-spectrum β -lactamases (ESBL) and *Klebsiella*

pneumoniae carbapenemase (KCP).(2) ESBL is an enzyme that hydrolyze penicillin, cephalosporin (first, second, third generations), oxymino- β -lactam compound (aztreonam, but not cephamycin and carbapenem) and may be inhibited by β -lactamase inhibitor (clavulanate, sulbactam and tazobactam).(3)

Various screening and confirmation tests have been developed to detect ESBL-producing *K. pneumoniae*. However, the increasing diversity of ESBL-producing *K. pneumoniae* strains, along with resistance mechanism of non-ESBL-producing *K. pneumoniae*, making identification of ESBL-producing *K. pneumoniae* by conventional phenotypic techniques difficult.(4) Double Disc Synergy Test (DDST) is able to find the phenotypic character profile of ESBL resistance against third generation cephalosporin (ceftazidim, cefpodoxime, ceftriaxone) and monobactams (aztreonam). The presence of ESBL-producing bacteria was indicated by an increase in inhibitory zone on amoxicillin/clavulanic acid through third-generation cephalosporins.(5)

There was a breakthrough in the development of selective chromogenic medium for the screening of ESBL-producing *K. pneumoniae* directly from clinical specimens. Selectivity was provided based on colony color by chromogenic media.(6) New ESBL media with chromogenic medium has been developed. It contains nutrient rich media with a mixture of antibiotics, including cefpodoxime for detection of ESBL-bearing bacteria.(7) A study reported that the new ESBL media had a sensitivity of 88% and specificity of 94.4% in detecting ESBL-producing *Enterobacteriaceae* in 24 hours.(8)

In detecting antimicrobial resistance, the role of routine laboratory services is very essential. Surveillance of bacterial pattern, antibiogram pattern, multiresistant bacterial detection is an important support for the treatment of patients with infections and reasonable antibiotic prescription. The objective of the study is to evaluate the sensitivity of new ESBL media with chromogenic medium in detecting ESBL-producing *K. pneumoniae*.

Methods

A diagnostic test study using 103 clinical isolates of *K. pneumoniae* was conducted. In this study, the ChromID® ESBL media (bioMérieux, Marcy-l'Étoile, France) was used as the new ESBL media with chromogenic medium. The ESBL media was independently and blindly compared to the DDST, the gold standard test in detecting ESBL-producing *K. pneumoniae*. DDST is known as one of the

confirmatory test (9) and has sensitivity of 91.3% and specificity of 100%.(10)

The clinical isolates of *K. pneumoniae* were obtained from urine, pus, blood, feces, sputum, cerebrospinal fluid, drain liquid, nasal sinus liquid, gastric wash, bronchi liquid, injury liquid and nasal swab. The clinical samples were inoculated on the appropriate media. After the growth was detected, identification of *K. pneumoniae* was performed using Vitek 2 System (bioMérieux).

The identified isolates of *K. pneumoniae* were inoculated on the ESBL media and incubated at 35°C for 18-24 hours, followed by observation and interpretation. The growth of green colonies were interpreted as ESBL-producing *K. pneumoniae*.

DDST was performed by inoculating 0.5 McFarland turbidity standard of *K. pneumoniae* suspension on the Mueller Hinton solid media plate. After the suspension was dried, 30 μ g ceftazidim, 30 μ g cefotaxime, 30 μ g cefepime and 20/10 μ g amoxycillin/clavulanic acid disc were added on the media. The media plates were incubated at 35°C for 18-24 hours. The presence/absence of an increase in inhibitory zone between β -lactam discs and β -lactam/ β -lactamase inhibitor disc can be observed after the incubation.

K. pneumoniae ATCC 7000603 was used as the positive control of ESBL-producing bacteria, whereas *Escherichia coli* ATCC 25922 was used as the negative control of non-ESBL-producing bacteria. The results of ESBL media and DDST were interpreted by two observers.

The collected data were analyzed using descriptive statistic. Diagnostic performance was evaluated using 2x2 table. The sensitivity, specificity, accuracy, positive predictive value, negative predictive value, positive likelihood ratio and negative likelihood ratio were determined with 95% confidence interval.

The study was conducted in Clinical Laboratory of Dr. Sardjito Hospital Yogyakarta, Indonesia started from July to September 2015. Ethical clearance was issued by the Medical and Health Research Ethics Committee Faculty of Medicine, University of Gadjah Mada, Yogyakarta, Indonesia No. KE/KF/868/EC/2015.

Results

Total number of *K. pneumoniae* isolates were 103, collected from patients with different ages, as shown in Table 1. Sources of isolates were mostly urine (29.1%), pus (28.2%) and blood (22.3%). Urine specimens were collected from patients diagnosed with urinary tractus infection, chronic

Table 1. Characteristics of sources of clinical isolates.

Characteristics of isolates	n	%
Number of isolates	103	100
Age		
Toddlers (<5 years)	34	33
Child (≥5 - <18 years)	10	9.7
Adults (≥18 - <55 years)	32	31.1
Elderly (≥55 years)	27	26.2
Source of isolates		
Urine	30	29.1
Pus	29	28.2
Blood	23	22.3
Feces	11	10.7
Cerebrospinal fluid	2	1.9
Sputum	1	1.0
Stool	1	1.0
Drain liquid	1	1.0
Nasal sinus liquid	1	1.0
Gastric wash	1	1.0
Bronchi liquid	1	1.0
Injury liquid	1	1.0
Nasal swab	1	1.0
Types of clinical care		
Intensive	19	18.5
Non-intensive: - Surgical	9	8.7
- Non Surgical	61	59.2
Outpatient	14	13.6

renal failure, acute renal failure and kidney stones. Pus specimens were collected from patients diagnosed with diabetic ulcers, mandibula abscess, post laparotomy, colon abscess and nasal cavity carcinoma. The patients were mostly from non-intensive care without surgical treatment (59.2%). Meanwhile, 18.5% of patients were taken care in intensive care unit. Detail characteristics of isolates regarding age, source of isolates and their respective ward were shown in Table 1.

Result of new ESBL media and DDST were shown in Table 2. DDST was able to detect 76 isolates of ESBL-producing *K. pneumoniae* (73.8%), whereas the ChromID® ESBL media was able to detect 83 isolates of ESBL-producing *K. pneumoniae* (80.6%). By using DDST as the standard, there were 9 samples with false positive and 2 samples with false negative results of new ESBL media.

Table 3 showed the test characteristic of ChromID ESBL media in detecting the ESBL-producing *K. pneumoniae*. New ESBL media had high sensitivity (97.4%). The media also had positive (89.2%) and negative (90%) predictive value.

Discussion

In this study, study subjects categorized as elderly (≥ 55 years old) was 26.2%. Meanwhile previous study in hemodialysis patients whom suffered from bacteremia caused by *K. pneumoniae* reported that the proportion of patients older than 65 years old was 57.8%. (11) Another study reported that majority of study subjects (54.2%) whom suffered from *K. pneumoniae* bloodstream infections were aged more than 60 years. (12)

Most of subjects in this study (68%) were from non-intensive care unit and only 18.5% were from the intensive care unit. A study in hemodialysis patients who also suffered from *K. pneumoniae* bacteremia, reported that 46.7% of subjects were from the intensive care unit. Another study reported that 67.9% of hospital-acquired *K. pneumoniae* infections were particularly among patients from neonatal intensive care unit, with mortality rate up to 70%. (13)

Reglier-Poupet stated that longer incubation period of new ESBL media test may improve the sensitivity of this test. Negative results obtained from the new ESBL media can be improved with longer incubation. Incubation for 24 hours was associated with sensitivity of 88% and specificity of 94.4%. Meanwhile, incubation for 48 hours resulted in sensitivity of 94% and specificity of 90.5%. In the new ESBL media test with a reincubation period for 48 hours, colonies still did not grow, indicating no ESBL-producing

Table 2. Comparison between ChromID ESBL media and DDST.

ChromID® ESBL Test		DDST		Total
		ESBL (+)	ESBL (-)	
ESBL (+)	ESBL (+)	74	9	83
	ESBL (-)	2	18	20
Total		76	27	103

Table 3. Test characteristic of ChromID ESBL.

	Value	95% CI
Sensitivity (%)	97.4	90.82 - 99.68
Specificity (%)	66.7	46.04 - 83.48
Positive Predictive Value (%)	89.2	80.35 - 94.95
Negative predictive value (%)	90	68.30 - 98.77
Positive Likelihood Ratio	2.9	1.71 - 4.99
Negative Likelihood Ratio	0.03	0.01 - 0.16

isolate.(8) In our current results, there were 2 samples with false negative result, although the media had been incubated for 48 hours. The colonies still did not grow, indicating no ESBL-producing isolate.(8) In this study, longer incubation period (reincubation) were not performed for false negative results. In these falsenegative results, patients actually had the disease, but results showed non-ESBL-producing *K. pneumoniae*.

In this study, there were 9 false positive results. These false positive results might be related with the antibiotic used in the new ESBL media test. There was only one type of antibiotic, cefpodoxime, while in DDST there were combinations of antibiotics including cefotaxime, amoxicilin, clavulanic acid. Therefore, false positive results could happened, whereas, shown as resistant results. The majority of ESBL enzymes, are different significantly in biochemical characteristics such as activity against specific β -lactam (e.g., cefotaxime, ceftazidime).(14) In false positive results, possibly there were non-*Enterobacteriaceae* isolates, *Enterobacteriaceae* strains with sefalosporinase, or penicillinase (*Klebsiella oxytoca*). The difficulty of the new ESBL media was to distinguish between ESBL-producing isolates and isolates that associated with other resistant mechanisms, i.e., strain mediated AmpC β -lactamase over production and strain with over production of β -lactamase in *K. oxytoca*.(8)

The new ESBL media test could be used for screening patients with clinical presentation that indicating a high suspicious of ESBL-producing bacteria. The new ESBL media could be an appropriate method for monitoring critically ill patients with ESBL carrier. Low specificity in this study, suggested that all suspected ESBL-producing bacteria that were tested with the new ESBL media should be confirmed with a confirmation test, for example DDST.(15)

In current study, this new ESBL media showed sensitivity of 97.4% and specificity of 66.7%. Another

study reported that the new ESBL media test had sensitivity of 94.9% and specificity of 94.9% in detecting ESBL-producing *Enterobacteriaceae*.(16)

Conclusion

The new ESBL media and DDST results were similar. The sensitivity, specificity, positive predictive value and negative predictive value of the new ESBL media were appropriate. Therefore, it could be used for screening patients with clinical presentation that indicating a high suspicious of ESBL-producing bacteria. All suspected ESBL-producing bacteria that were tested with the new ESBL media should be confirmed with a confirmation test, such as DDST.

Acknowledgement

We would like to thank the Director of Dr. Sardjito Hospital who has given permission to conduct this study. Our gratitude also goes to the Chair of The Department of Clinical Pathology and Laboratory Medicine, to the Chair of Clinical Laboratory of Dr. Sardjito Hospital, together with the staffs whom supported the study, as well as to the patients who have been willing to participate as the research subjects.

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