RESEARCH ARTICLE

Sensitivity of ChromID® ESBL to Detects an Extended Spectrum β-Lactamases Producing *Klebsiella pneumoniae*

Emy Noerwidayati[1], Andaru Dahesihdewi[2], Osman Sianipar[3*]

[1,2,3]Department of Clinical Pathology and Laboratory Medicine, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, 55284, Indonesia

*Coresponding author. E-mail: osmansianipar@ugm.ac.id
Phone: 62-274-867421

Abstract

INTRODUCTION: *Klepsiella pneumoniae* is one of clinical isolates that frequently found as the causative agent of hospital aquired infection. Currently, most of *K. pneumoniae* are found to be extended-Spectrum β-lactamases (ESBL) producer so that to become multidrug resistant. A clinical laboratory with limited facilities need a valid, reliable, inexpensive, and simple laboratory test in order to control the infection as well as antimicrobial resistant. The objective of this study is to evaluate the diagnostic performance of ChromID® ESBL media to detect ESBL producing *K. pneumoniae*.
METHODS: This was a diagnostic test study where the ChromID® ESBL media was independently and blindly compared with the double disc synergy test to detect clinical isolate of ESBL-producing *K. pneumoniae*. Subjects the study were clinical isolates of *K. pneumoniae* found in the Clinical Laboratory of Dr. Sardjito Hospital which were isolated from clinical samples. Clinical data were obtained from a patient's medical records. The data were analyzed using descriptive statistics and 2x2 tables to calculate diagnostic performance.
RESULTS: There were 103 clinical isolates of *K. pneumoniae* which were isolated from a sample of urine, pus, blood, stool, cerebrospinal fluid, sputum, etc. Number of both true positive and true negative result were 92, and it was found 9 false positive and 2 false negative. The ChromID® ESBL media showed sensitivity, specificity, positive predictive value, negative predictive value, likelihood ratio for positive result, and likelihood ratio for negative result were 97.4%, 66.7%, 89.2%, 90%, 2.9 and 0.03 respectively.

CONCLUSION: It colud be concluded that the media ChromID® ESBLcould be used to detect a clinical isolates of ESBL-producing *K. pneumoniae*.

KEYWORDS: Sensitivity, ChromID® ESBL, DDST, ESBL, *K. pneumoniae*

Abstrak

LATAR BELAKANG: *Klepsiella pneumoniae* merupakan salah satu isolat klinis yang sering ditemukan sebagai penyebab infeksi yang didapat di rumah sakit. Saat ini sebagian besar *K. pneumoniae* ditemukan sebagai penghasil enzim *extended-spectrum β-lactamases* (ESBL) sehingga bersifat multi resisten. Laboratorium klinik dengan fasilitas yang terbatas memerlukan suatu pemeriksaan yang tepat, teliti, murah, dan sederhana dalam rangka pengendalian infeksi dan resistensi antimikrobia. Penelitian ini bertujuan untuk mengevaluasi kemampuan diagnostik media ChromID® ESBL untuk mendeteksi *K. pneumoniae* penghasil ESBL.

METODE: Penelitian ini merupakan penelitian uji diagnostik dimana media ChromID® ESBL dibandingkan dengan *double disc synergy test* (DDST) secara bebas dan tersamar untuk mendeteksi isolat klinis *K. pneumoniae* penghasil ESBL. Subyek dalam penelitian ini adalah isolat klinis *K. pneumoniae* yang didapatkan di Instalasi Laboratorium Klinik RSUP Dr. Sardjito Yogyakarta dari berbagai sampel klinis. Data klinis pasien didapat dari catatan medis pasien. Data yang diperoleh dianalisis menggunakan statistik deskriptif dan tabel 2x2 untuk menghitung kemampuan diagnostik.

HASIL: Total isolat klinis *K. pneumoniae* yang didapat sebanyak 103 isolat yang didapat dari sampel urin, pus, darah, feces, cairan spinal, sputum, dan lain-lain. Jumlah positif benar dan negatif benar sebanyak 92. Hasil positif palsu sebanyak 9 dan negatif palsu sebanyak 2. Media ChromID® ESBL menunjukkan sensitifitas, spesifisitas, nilai ramal positif, nilai ramal negatif, rasio kemungkinan untuk tes positif, dan rasio kemungkinan untuk test negatif secara berturut-turut sebesar 97,4%, 66,7%, 89,2%, 90%, 2,9 dan 0,03.

KESIMPULAN: Media ChromID® ESBL dapat digunakan untuk mendeteksi isolat klinis *K. pneumoniae* penghasil ESBL.

KATA KUNCI: Sensitifitas, ChromID® ESBL, DDST, ESBL, *K. pneumoniae*

Introduction

In developing countries, infectious diseases are still in the first place for causes of illness in community. Treatments for infections usually involve antibiotics. With advances in technology, the number and type of clinically useful antibiotics is increasing, resulting in requirement for high precision in choosing an antibiotic. Inappropriate antibiotic treatment may have adverse effects, i.e. the emergence of bacterial resistance and low antibiotic efficacy against certain bacteria. Bacterial resistance against antibiotics are critically important.[1]

Typically, this resistance is caused by infections that develop in hospitals (nosocomial infections). Many bacteria cause infections, *Klebsiella spp.* is one out of them. It is an important pathogenic bacteria in nosocomial infections. Generally, *Klebsiella spp* infections are implicated in pneumonia, urinary tractus infections, meningitis and septicemia. Increased resistant of *Klebsiella spp.* has been widely reported as the effect of enzymes extended Spectrum β-lactamases (ESBL) and *Klebsiella pneumoniae Carbapenemase* (KCP).[1] Extended Spectrum β-lactamases (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).[2]

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 the non-ESBL producing *K. pneumoniae* resistance mechanisms, have caused overlapping resistance phenotypes, thus making identification of ESBL producing *K. pneumoniae* by conventional phenotypic techniques difficult.[3] Performance of bacterial phenotypic tests (disc diffusion and microdilution methods) was made according to the Clinical and Laboratory Standards for Antimicrobial Susceptibility

Testing recommendations. Double disc synergy test found characteristic phenotypic profile of ESBL resistance against 3rd 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.[4]

More recently, there was a breakthrough in the development of better selective chromogenic medium to improve screening for ESBL producing *K. pneumoniae* directly from clinical specimens. Selectivity is provided with a mixture of antibiotics and identification of microorganisms based on colony color by a chromogenic media.[5,6] ChromID® ESBL media is a new and innovative chromogenic medium, designed specifically for ESBL-producing bacteria screening. Isolation and detection of ESBL is based on the content of a nutrient rich media with a mixture of antibiotics, including cefpodoxime. This antibiotic is recognized as a marker of choice for this resistance mechanism, and can inhibit selectively against Gram-positive bacteria and fungi.[7] ChromID® ESBL media is a convenient method, inoculation can be made directly from clinical sample, results are obtained relatively fast 18 -24 hours after incubation, it is relatively low cost and can be applied in the laboratory with limited resources are limited. A study by Glupczynski *et al.,* (2007) found that ESBL detection using ChromID® ESBL media had a sensitivity (97.3%) and specificity (90,4%).

The role of routine laboratory services in clinical microbiology for the detection of antimicrobial resistance is very important. Surveillance of bacterial pattern, antibiogram pattern, multiresistant bacterial detection by clinical laboratories 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 ChromID® ESBL media in detecting ESBL-producing *K. pneumoniae.*

Methods

This was a diagnostic test study which involved 103 clinical isolates of *K. pneumoniae*. In this study the ChromID® ESBL media was independently and blindly compared with DDST as the reference standard test, in order to detect ESBL producing *K. pneumoniae*.

The clinical isolates of *K. pneumoniae* were obtained from clinical sample of urine, pus, blood, feces, sputum, cerebrospinal fluid, etc. The clinical samples were inoculated onto appropriate media, and after the growth detected then followed by identification. Identification of *K. pneumoniae* was performed using Vitek 2 system. These clinical isolates of *K. pneumoniae* then were tested with the ChromID® ESBL media and DDST.

The ChromID® ESBL media were performed as follows: inoculate a colony of *K. pneumoniae* onto ChromID® ESBL media, and incubate it at 35°C for 18-24 hours. After incubation completely accomplished then follwed by observation and interpretation. The growth of green colonies were interpretate as ESBL producing *K. pneumoniae*.

Double disc synergi test was performed as follows: inoculated 0.5 McFarland turbidity standard of suspension of *K. pneumoniae* onto Mueller Hinton solid media plate and let it dried. Then put onto this media plate the antimicrobial discs namely ceftazidim (30 µg), cefotaxime (30 µg), cefepime (30 µg), and amoxycillin/clavulanic acid disc (20/10 µg). Placed amoxycillin/clavulanic acid disc in the center, and placed the other 3 antimicrobial disc with 20 mm distance from the amoxycillin/clavulanic acid disc. Incubated the media plate at 35°C for 18-24 hours, and then read the presence/absence of an increase in inhibitory zone between β-lactam discs and β-lactam/β-lactamase inhibitor disc. ESBL-producing *K. pneumoniae* was identified whenever there was reduced-sensitivity to β-lactam antibiotic discs along with the presence of synergy between at least 1 of 3 β-lactam antibiotic discs (cefotaxime, cefepime, ceftazidim,) and amoxilin/clavulanic acid with clear shadows outside inhibition zone. Non-producing ESBL *K. pneumoniae* was identified whenever there is

increased sensitivity to β-lactam antibiotic discs and lack of synergy between at least 1 of 3 β-lactam antibiotic discs (cefotaxime, cefepime, ceftazidim,) and amoxilin/clavulanic acid without clear outside inhibition zone.

Factors that may affect the tests, including media (pH, thickness, moisture), inoculum and incubation time were appropriate controled. *Klepsiella pneumoniae* ATCC 7000603 was used as the positive control of ESBL producing bacteria, whereas *E. coli* ATCC 25922 was used as the negative control of non-ESBL producing bacteria. Readings between two observers for DDST and ChromID® ESBL media in interpreting the results of ESBL (+) and ESBL (-), had an Kappa index of 1, it indicated a perfect agreement.

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

The study was conducted in Clinical Laboratory of Dr. Sardjito Hospital Yogyakarta, Indonesia sttarted from July up to September 2015. Ethical clearance was issued by the Medical and Health Research Ethics Committee Faculty of Medicine, University of Gadjah Mada, Yogyakarta, Indonesia Ref: KE/KF/868/EC/2015.

Results

Sources of *K. pneumoniae* isolates were mostly from urine (29.1%), and followed by pus (28.2%), blood, feces, sputum, serebrospinal fluid. Other sources of isolates includes drain liquid (1 sample), nasal sinus liquid (1 sample), gastric wash (2 samples), bronchi liquid (1 sample), injury liquid (one sample), nasal swab (1 sample). Isolate source of urine were collected from patients diagnosed with urinary tractus infection, chronic renal failure, acute renal failure, and kidney stones. Isolate source of pus were collected from patients diagnosed

Table 1. Characteristics of study 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:		
• Blood	23	22.3
• Pus	29	28.2
• Urine	30	29.1
• Feces	11	10.7
• Sputum	1	0.9
• Cerebrospinal fluid	2	1.9
• Etc	7	6.8
Types of clinical care		
• Intensive	19	18.5
• Non-intensive: - Surgical	9	8.7
- Non Surgical	61	59.2
• Outpatient	14	13.6

with diabetic ulcers, mandibula absces, post laparotomy, colon absces and nasal cavity carcinoma. A study by Noviana (2004), in a private hospital in Jakarta, found the most common source of clinical clinical sampel were urine 40.3%, and pus 37.1%. This study found that 18.5% of patients were taken care in intensive care unit. Gupta *et al.,*(2003) found that 67.9% of hospital acquired infections caused by *K. pneumonia* particularly among patients in neonatal intensive care unit with mortality rate of up to 70%. Characteristics of isolates regarding age, source of isolates, and ward were shown in table 1.

Compararative data between the ChromID® ESBL media and DDST were presented in table 2. From this table, DDST was able to detect 76 isolates of *K. pneumoniae* producing ESBL (73.8%), whereas the ChromID® ESBL media was able to detect 83 isolates of *K. pneumoniae* producing ESBL (80.6%). There were 9 ESBL samples with false positive results, positive ESBL results from ChromID® ESBL media but negative ESBL results with DDST. These false positive results may be caused by only one type of antibiotic

used in ChromID® ESBL media which is cefpodoxime, while in DDST, cefotaxime, amoxicilin/clavulanic acid were used. Strains that are sensitive to antibiotic cefpodoxime,

Table 2. Comparison between ChromID® ESBL media and DDST

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

when tested with antibiotics cefepime, ceftazidime, cefotaxime, amoxcilin/clavulanic acid may show resistant results. Detection of ESBL reflects the level of resistant shown by strains expressing enzyme in vivo. The majority of ESBL enzymes, are different significantly in biochemical characteristics such as activity against specific β-lactam (eg, cefotaxime, ceftazidime).[11] In false positive results, a confirmatory test is required to ensure ESBL producing *K. pneumoniae* and to exclude non-*Enterobacteriaceae* isolates, *Enterobacteriaceae* strains with sefalosporinase, or penicillinase *(K. oxytoca)* overproduction. The difficulty of ChromID® ESBL media was to distinguish between ESBL producing isolates and isolates that associated with other resistant mechanisms, i.e. strain mediated *AmpC* beta-lactamase over production and strain with over production of beta-lactamase in *Klebsiella oxytoca,* thus these strains may give a false positive result on this ChromID® media.[12] In the false-positive results, patients did not actually have it, but the results showed positive results for ESBL producing *K. pneumoniae*, so these patients were administered with antibiotic treatment for ESBL *K. pneumoniae* infections. Inappropriate antibiotic therapy may lead to additional costs both for patients and their families. Antibiotics typically used in patients with ESBL *K. pneumoniae* infections is carbapenem. Carbapenem antibiotic treatment is costly, this not including maintenance costs, administration costs, doctor's fees and cost of diagnostic studies for treatment evaluation. Treatment with

Carbapenem antibiotics that are not in accordance with these indications may also result in resistant problem.

There were two ESBL samples with false negative result, i.e. negative ESBL results with ChromID® method, but positive results with DDST. Negative results obtained from ChromID® ESBL media can be improved with longer incubation period beyond 24 hours, in order to optimize the sensitivity. A study by Reglier-Poupet (2008) found that longer incubation period ChromID® ESBL test may improve the sensitivity of this test. 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 ChromID® ESBL test with a reincubation period for 48 hours, colonies still did not grow, indicating no ESBL producing isolate. In this study, longer incubation period (reincubation) were not performed for false negative results. In these false-negative results, patients actually had the disease, but results showed non-ESBL producing *K. pneumoniae*. Patients who were infected with Kp-ESBL but did not receive appropriate treatment, may had increased morbidity and mortality.

Table 3 show the test characteristic of ChromID® ESBL to detect ESBL producing *K. pneumoniae*. It had sensitivity of 97.4% meaning that this test had ability to detect 97.4% of ESBL producing *K. pneumoniae*. The test also had specificity of 66.7%. It reflected that the test was able to detect 66.7% of non-ESBL producing *K. pneumoniae*. High sensitivity

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

of ChromID® ESBL media suggested that this media was an excellent method for screening for ESBL producing *K. pneumoniae*. The ChromID® ESBL media could be used in situations of ESBL outbreaks, for example for screening patients with clinical presentation that indicating a high suspicious of ESBL producing bacteris. The ChromID® ESBL media was an appropriate method for monitoring patients with ESBL carrier status in critically ill patients. Low specificity in this study, suggested that all suspected ESBL producing bacteria that were tested with ChromID® ESBL media should be confirmed with a confirmation test for example DDST.[13]

Conclusion

The ChromID® ESBL had sensitivity, specificity, positive predictive value, negative predictive value, likelihood ratio for positive test, and likelihood ratio for negative test were 97.4%, 66.7%, 89.2%, 90%, 2.9 and 0.03 respectively. Therefore, it colud be used to detect ESBL-producing *K. pneumoniae*

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Refferences

1. Jawetz E. Antimicrobial Chemotherapy. In : Brooks GF, Butel JS, Morse SA, editors. Medical Microbiology, 22nd ed, Appleton &Lange, California, 2013.
2. EUCAST. EUCAST Guidelines for Detection of Resistance Mechanisms and Specific Resistances of Clinical and/or Epidemiological Importance: *Extended Spectrum β-Lactamases Producing Enterobacteriaceae*. 2012; p. 11-23.
3. Lucet JCD, Decre A, Fichelle ML, Joly-Guillou M, Pernet C, Deblangy MJ, Kosmann B., Regnier. Control of a Prolonged Outbreak of Extended-Spectrum β-Lactamase-Producing Enterobacteriaceae in a university hospital. Clin Infect Dis. 1999; 29:1411–1418.
4. Fatima N, Mirsada H, Zineta D, Nijaz T, Selma P, Merima G. Comparison of Double Disk Synergy Test, Vitex 2 and Check-MDR CT102 for Detection of ESBL Producing Isolats. Acta Med Acad. 2012; 42 (1): 15-24.

5. Diederen BMW, Van Leest ML, Van Duijn I, Willemse P, Van Keulen PHJ, Kluytmans JAJW. Performance of MRSA ID: a New Chromogenic medium for Detection of Methicillin-Resistant *Staphylococcus aureus*. J Clin Microbiol. 2006; 44(2): 586–588.
6. Meyer E, Serr A, Schneider C, Utzolino S, Kern WV, Scholz R, Dettenkofer M. Should We Screen Patients for Extended Spectrum β-Lactamase-Producing Enterobacteriaceae in Intensive Care Units. Infect. Control Hosp. Epidemiol. 2009; 30:103–105.
7. Vasough M. An Article on Chromogenic Media: Facts about Micro & Molecular Biology. 2010. Diunduh dari: *http//www.condolab.com.* (acessed on September 2, 2015)
8. Glupczynski Y, Berhin C, Bauraing C, Bogaerts P. Evaluation of a New Selective Chromogenic Medium for Detection of Extended Spectrum β-Lactamases Producing Enterobacteriaceae. J Clin Microbiol. 2007; 4:501-505.
9. Noviana H. Pola Kepekaan Antibiotika *Escherichia coli* yang Diisolasi dari Berbagai Spesimen Klinis. Jurnal Kedokteran Trisakti. 2004; 23(3): 122-126.
10. Gupta A, Ampofo K, Rubenstein D, Saiman L. Extended Spectrum β-Lactamases-Producing K. pneumonia Infections: a Review of the Literature. J Perinatology. 2003; 23, 439-443.
11. Sturenburg E, Mack D. Extended-Spectrum β-Lactamase: Implications for the Clinical Microbiology Laboratori, Therapy and Infection Control. J Infec. 2003; 47:273-295.
12. Reglier-Poupet H, Naas T, Carrer A, Cady A, Adam JM, Fortineau N, Poyart C, Nordmann P. Performance of ChromID ESBL, a chromogenic medium for detection of Enterobacteriaceae producing extended-spectrum β-Lactamases. J Med Microbiol. 2008; 57: 310-315.
13. Farber J, Moder KA, Layer F, Tammer I, Konig W, Konig B. Extended-Spectrum β-Lactamase Detection with Different panels for Automated Susceptibility Testing and with a Chromogenic Medium. J Clin Microbiol. 2008; 46(11): 3721-3727.