Evaluation of Francis media for extended spectrum beta lactamase (ESBL) screening

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Francis media was developed for the differential screening of Burkholderia pseudomallei. It was later found to have additional function of selecting extended spectrum beta lactamase (ESBL) from Enterobacteriaceae. A total of 305 Enterobacteriaceae isolates from clinical specimens (Klebsiella spp. [191], Escherichia coli [96], Enterobacter spp. [9], Citrobacter spp. [3] and others [6]) were tested for the presence of ESBL. Out of 305, 135 were ESBL producing Enterobacteriaceae tested on Francis media for the presence of yellow colonies and haze after 24 h of incubation. Francis media revealed sensitivity and specificity of 89 and 99%, respectively in detecting ESBL producing Enterobacteriaceae.

Key words: Screening, extended spectrum beta lactamase (ESBL), Francis media.

INTRODUCTION

Extended spectrum beta lactamase (ESBL) is one of the multidrug resistant (MDR) organisms under constant surveillance in Malaysian hospitals. Due to its ability to spread rapidly in the hospital environment, these bacteria tend to cause nosocomial outbreaks and increase the length of hospitalization (Sturenburg and Mack, 2003; Kim et al., 2002; Paterson et al., 2001). Early detection and identification of ESBL is important to optimize antimicrobial therapy and to ensure timely introduction of appropriate infection control procedures (Cantón and Coque, 2006; Pfaller and Segreti, 2006; Ramphal and Ambrose, 2006).

Screening and confirmation of ESBLs are normally performed on isolated organisms following microscopic examination of Gram negative rods (GNR) and culture on MacConkey and antibiotic susceptibility testing. Most times, there is a delay of 18-24 h before ESBL presence could be identified phenotypically from a primary culture. At this juncture, there is a need of rapid screening for carriage of ESBL producing Enterobacteriaceae among high-risk patients by using a highly selective media. Until date, there are few commercial selective ESBL media been evaluated and produced (Glupczynski et al., 2007; Huang et al., 2010; Paniagua et al., 2010; Reglier-Poupet et al., 2008; Saito et al., 2010).

Francis media was developed for the differential screening of Burkholderia pseudomallei and Burkholderia cepacia (Francis et al., 2006). This media which uses gentamicin as its inhibitory components, was later found to have additional function of selecting ESBL producing isolates. In this work, we reported an evaluation of Francis media in selecting ESBL producing...
Figure 1. ESBL producing Enterobactericeae on Francis media.

Figure 2. K. pneumoniae appearance of yellow colonies with yellow discoloration of Francis media.

Enterobactericeae from clinical isolates.

MATERIALS AND METHODS

A total of 305 clinical specimens, sent to our microbiology laboratory for culture and sensitivity were included in the study. These specimens included blood, pus (wound swabs or ear-nose-throat specimens), tissue, urine and stool, originating from 279 patients who had been hospitalized in various medical and surgical wards for more than 48 h. All blood specimens were isolated using the BD BACTECTM blood culture system. Each specimen that was identified as Gram negative rods on microscopic examination was directly inoculated onto horseblood agar and MacConkey (Oxoid, Basingstoke, United Kingdom) and incubated in air at 37°C for 18 to 24 h.

Gram negative colonies were inoculated unto Francis media (prepared as in Francis et al., 2006) and homogenized in 1 ml of sterile physiological saline (0.85%) into 0.5 McFarland suspension and plated spirally onto Muller Hinton agar with antibiotic disc (30 µg Cefotaxime, 10 µg Amoxicillin-clavulanic acid and 30 µg Ceftazidime placed 1.5 cm distance in a row) for ESBL screening and incubated at 35°C for 18 to 24 h. Growth on Francis agar is linked to the resistance to a specific antibiotic and signified by presence of yellow colonies and yellow discoloration of Francis agar were regarded as ESBL isolates (Figure 1). Positive screening of ESBL isolates on Muller Hinton agar were reflected by the presence of ‘keyhole effect’ between the discs after incubation and subjected to confirmation of ESBL-producing isolates by combined double disks (30 g ceftazidime and 30 g cefotaxime disks alone and the same antimicrobials with 10 g clavulanic acid) as recommended by CLSI guidelines (CLSI, 2011).

All culture plates were interpreted independently by two laboratory staff members. Pronounced yellow haze around the colonies on the Francis agar signified a presumptive ESBL organism (Figure 2). All types of colonies presenting different morphological aspects were identified by API 20NE and API 20E (bioMe’rieux, Marcy l’Etoile, France). The plates were incubated in ambient air at 37°C for 18 to 24 h. Genotypic characterization of resistance mechanisms was determined by PCR assays targeting \( \text{bla}_{\text{TEM}}, \text{bla}_{\text{SHV}} \) and \( \text{bla}_{\text{CTX-M}} \) genes according to previously published methods (Sidjabat et al., 2009).

RESULTS AND DISCUSSION

Overall, 305 isolates were identified as Gram negative rods on microscopic examinations. They were screened for Enterobactericeae group by using simple TSI sugar sets. The isolates recovered included Klebsiella spp. [191], Escherichia coli [96], Enterobacter spp. [9], Citrobacter spp. [3] and other Enterobactericeae [6] (Table 1). Out of this total of 305 Enterobactericeae isolated, 135 were confirmed as ESBL isolates by the four disk method as mentioned earlier. These 135 isolates when streaked onto Francis media, 120 (89%) isolate yielded yellow colonies with yellow haze around the colonies after 24 h of incubation. However 11% (15/135) isolates did not produce yellow coloration on Francis agar after 24 h of incubation. Among the 15 false negative ESBL isolates, four Klebsiella pneumonia isolates yielded pale colored colonies, and the remaining 11 isolates (1 Enterobacter aerogenes, 1 Enterobacter cloacae and 9 E. coli) did not grow on the agar, as they were sensitive to gentamicin. Only two isolates of K. pneumonia showed false positive results, as they were resistant to gentamicin as shown by disk diffusion test for these isolates. They produced pale yellow colouration probably due to incomplete fermentation of the sugars present in the media.

Prolongation of incubation did not increase the sensitivity of detection of ESBL-producing organisms on the media and only marginally increased the growth of other Gram-negative isolates. Molecular typing of ten randomly selected isolates was done. The ESBLs was performed by PCR analysis for \( \beta \)-lactamase genes which showed all 5 isolates possessed the \( \text{bla}_{\text{SHV}}, \text{bla}_{\text{TEM}} \) and \( \text{bla}_{\text{CTX-M-15}} \) cluster gene.

Overall, we showed that Francis media was a reliable culture media to screen for ESBL-producing Enterobactericeae directly from clinical samples. The
Table 1. Detection of ESBL and Non-ESBL organisms using Francis media as compared to CLSI recommended ESBL confirmatory disk diffusion method.

<table>
<thead>
<tr>
<th>ESBL±</th>
<th>Francis (n)</th>
<th>CLSI (n)</th>
<th>Non-ESBL* (n)</th>
<th>Francis (n)</th>
<th>CLSI (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterobacter cloacae</td>
<td>0</td>
<td>1</td>
<td>Acinetobacter baumannii</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Enterobactera aerogenes</td>
<td>2</td>
<td>3</td>
<td>Citrobacter freundii</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>6</td>
<td>15</td>
<td>Enterobacter aerogenes</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Citrobacter freundii</td>
<td>1</td>
<td>1</td>
<td>Enterobacter agglomerans</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Klebsiellapneumoniae</td>
<td>110</td>
<td>114</td>
<td>Enterobacter cloacae</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Klebsiellaoxytoca</td>
<td>1</td>
<td>1</td>
<td>Escherichia coli</td>
<td>81</td>
<td>81</td>
</tr>
<tr>
<td>Flavobacterium spp.</td>
<td>1</td>
<td>1</td>
<td>Klebsiella pneumoniae</td>
<td>69</td>
<td>71</td>
</tr>
<tr>
<td>Klebsiella oxytoca</td>
<td>5</td>
<td>5</td>
<td>Proteus mirabilis</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Proteus vulgaris</td>
<td>1</td>
<td>1</td>
<td>Pseudomonas aeruginosa</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Total | 120 | 135 | Total | 168 | 170 |

Sensitivity (%) | 89 | NA | Specificity (%) | 99 | NA |
PPV(%) | 98 | NA | NPV (%) | 92 | NA |

±Number of ESBL isolates correctly screened by Francis media and confirmed by CLSI method;
*Number of non-ESBL isolates correctly screened by Francis media and confirmed by CLSI method;
^SHV, TEM and CTX-M-15 were found on these ESBLs isolated from Francis media.

The main advantage of this media is its significant higher specificity (99%) after 24 h incubation. This was due to lower recovery of non-ESBL producing Enterobacteriaceae and less false-positive results due to non-Enterobacteriaceae. Thus, it reduces the need for further ESBL confirmation testing and unnecessary identification work when disregarding all colonies without a yellow colonies and yellowish discoloration of the agar. Yielding an excellent positive predictive value (98%), this media enable rapid identification of patients carrying ESBL-producing Enterobacteriaceae.

Most ESBL producing Enterobacteriaceae are noted to have co-resistance effect with aminoglycoside (Winokur et al., 2001; Obeng et al., 2013). This explains why in this study all 135 ESBL producing Enterobacteriaceae were able to grow in the Francis media which contains gentamicin. The Enterobacteriaceae carry out fermentation reaction on the complex sugars present causing the media to turn acid causing a profound yellow colouration on Francis media. The main limitation of Francis media was noted in ESBL detection in other isolates apart from K. pneumonia strains. This was noted in isolates with lower inoculum and particularly affected strains with pronounced substrate and supplement preference in the media. As ESBL enzymes have also become more prevalence among species with an inducible AmpC type β-lactamase such as Enterobacter spp. and Citrobacter freundii, interpretation of positive findings need to be performed cautiously as resistant phenotypes other than ESBL (the AmpC producers) may demonstrate similar phenotype as ESBL on Francis media.

From this study, Francis media showed high specificity and sensitivity as other reports of ESBL screening media (Hadziyannis et al., 2000; Huang et al., 2010; Sturenburg et al., 2005). However, the drawback in this study was limited strains of ESBL-producing Enterobacteriaceae as the samples were collected from one centre. A more comprehensive study is deemed important to evaluate its usefulness and to further improve its formulation as an ESBL screening media. Nevertheless, Francis media can be an alternative for a cheap routine ESBL screening to prompt the initiation of infection control measures.

Conflicts of Interests

The author(s) have not declared any conflict of interests.

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References


