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Evaluation of chromID OXA-48 for the recovery of carbapenemase-producing *Enterobacteriaceae* from rectal swabs from hospitalized patients in Ankara, Turkey

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Introduction

Background: There is a pressing need to define robust standardized screening methods for the effective detection of carbapenemase-producing *Enterobacteriaceae* (CPE) in order to control their spread.[1] To address this need the Centers for Disease Control recommended a broth enrichment method that could be used in almost any laboratory.[2] This method entails the inoculation of a rectal swab into 5 ml of trypticase soy broth (TSB) to which a 10 µg carbapenem disc has been added (meropenem or ertapenem). The broth is then subcultured after overnight incubation at 37°C onto MacConkey agar. However, it is increasingly recognized that chromogenic culture media may have a useful role to play in the efficient detection of CPE.[3,4]

Purpose of the Study: The aim of this study was to critically assess three culture methods for screening hospitalized patients in Ankara, Turkey, for potential gut colonization with CPE. These three methods comprised: enrichment culture using TSB plus 2 mg/L ertapenem as recommended by CDC; direct culture on an established chromogenic agar designed for detection of CPE (chromID CARBA) and, direct culture on a chromogenic agar specifically designed for isolation of CPE that produce OXA-48 carbapenemase (chromID OXA-48).

Materials and Methods

Rectal swabs were obtained from 302 distinct patients as part of routine screening for CPE. Material from swabs was suspended in 0.5 ml sterile saline (0.85%) and 50 µl inoculated onto chromID OXA-48, chromID Carba and 5mL TSB containing a 10µg ertapenem disc. All media were incubated for 18-20 h at 37°C. After incubation, 10 µL of broth was inoculated onto MacConkey agar and incubated overnight at 37°C. All isolates were identified by MALDI-TOF MS. Any *Enterobacteriaceae* isolated on any of the three media were screened for possible carbapenemase production in accordance with UK national guidelines using the KPC, MBL & OXA48 confirm ID kit (Rosco) and confirmed using PCR for the five most common carbapenemase genes (OXA-48, KPC, VIM, IMP and NDM-1).

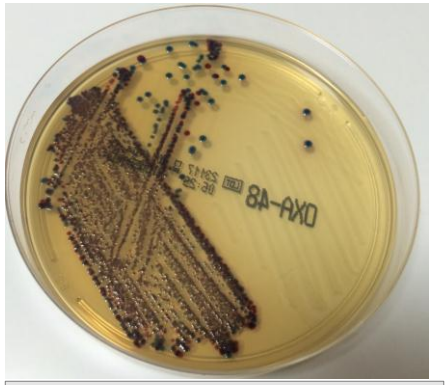

Fig.1. *K. pneumoniae* (green) and *E. coli* (red) - both with OXA-48 carbapenemase - cultured on chromID OXA 48.

References

1. Voulgari E, Poulou A, Koumaki V, Tsakris A. 2013. Future Microbiol. **8:**27-39. **2. Centers for Disease Control and Prevention.** 2009. http://www.cdc.gov/HAI/pdfs/labSettings/Klebsiella_or_Ecoli.pdf. **3. Vrioni G, Daniil I, Voulgari E, *et al*** 2012. J. Clin. Microbiol. **50:**1841-1846. **4. Girlich D, Poirel L, Nordmann P.** 2013. Diagn. Microbiol. Infect. Dis. **75:**214-7.

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Results

TABLE 1: Total number of colonized patients detected by each method and by combinations of methods.

	n	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Total	33				
CDC method	19	57.6	95.2	59.4	94.8
chromID OXA-48	25	75.8	99.3	92.6	97.1
chromID Carba	19	57.6	98.9	86.4	95
chromID OXA-48 plus CDC method	30	90.9	94.8	68.2	98.8
chromID OXA-48 plus chromID Carba	30	90.9	98.5	88.2	98.9
chromID Carba plus CDC method.	25	75.8	94.4	62.5	96.9

A total of 33 patients (11%) were found to be colonized with CPE out of 302 distinct patients who were screened. *Klebsiella pneumoniae* was by far the most prominent species of CPE and was isolated from 31 of the 33 colonized patients. All isolates of CPE were confirmed as harboring OXA-48 carbapenemase as confirmed by both phenotypic testing and PCR. No other carbapenemases were detected in the isolates of *Enterobacteriaceae*. Table 1 shows the sensitivity of each method (and combinations of the three methods) for detection of colonized patients.

Direct culture of stool samples onto chromID OXA-48 was the most sensitive single method in this setting (sensitivity: 75.8%; $P = 0.2$) and the use of a combination of chromogenic media increased the sensitivity to 90.9%. Use of the CDC broth enrichment method resulted in a relatively poor positive predictive value (due to the recovery of 18 false positives) and had the disadvantage of requiring 2 days for isolation of colonies.

Conclusions:

✓chromID OXA-48 was the best single method for screening for CPE in this setting where only OXA-48 producers were encountered, and was more sensitive than the CDC broth method.

✓A combination of chromID OXA-48 with chromID CARBA offered isolation of presumptive carbapenemase-producing *Enterobacteriaceae* within 18-20 h with high sensitivity (90.9%) and specificity (98.5%). All CPE recovered on either chromogenic medium formed coloured colonies allowing easy discrimination from other organisms e.g. *Acinetobacter* species.