Evaluation of Chromatic™ OXA-48 medium for direct detection of OXA-48 producing *Enterobacteriaceae* from rectal swabs

A. Antonelli[1,2], L. Henrici De Angelis[1], J. Tyrrell[2], T. Walsh[2], D. Vitagliano[3], F. Brocco[3], G. M. Rossolini[1,4,5] and M. M. D'Andrea[1]

[1]Department of Medical Biotechnologies, University of Siena, Siena, Italy; [2]Department of Medical Microbiology & Infectious Diseases, Cardiff University, Heath Park, Cardiff, Wales, UK. [3]Liofilchem s.r.l, Scientific Division, Roseto degli Abruzzi, Italy; [4]Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy; [5]Clinical Microbiology and Virology Unit, Careggi University Hospital, Florence, Italy.

Background

Rapid identification of patients colonized by Carbapenem-Resistant *Enterobacteriaceae* (CRE) is a crucial step for both epidemiological surveillance and infection control measures. In fact CRE colonization often precedes infection, and carriers are also a major source of dissemination of CRE in the hospital setting. Among CRE there is an increasing trend of isolation of OXA-48 producers in several Mediterranean and European countries such as Turkey, France, Germany, Spain, the Netherlands and the United Kingdom. In Italy, even if KPC-producers are the most commonly found CRE in hospital settings, sporadic cases of OXA-48 producing CRE have been reported and this fact warrants continuous surveillance to control the spread of this resistance mechanism. In this work we evaluated the performance of a new selective chromogenic medium for the rapid detection of patients colonized by OXA-48 producing CRE.

Table 1. Species distribution of the reference strains tested during Phase II

Species	Number	%	Carbapenem-resistance mechanisms
Klebsiella spp.	20	40	VIM, NDM, OXA-48-like, KPC, DP[a]
Escherichia coli	13	26	NDM, OXA-48-like, OXA-24-like, KPC
Citrobacter freundii	3	6	OXA-48-like, KPC, OXA-372
Enterobacter cloacae complex	9	18	VIM, NDM, IMI-2, NMC-A
Proteus mirabilis	3	6	KPC-2
Serratia marcescens	1	2	NDM-1
Shewanella xiamenensis	1	2	OXA-416

[a] DP: Defect of permeability associated with the production of CTX-M type enzymes

Table 2. Experimental results of reference strains plating

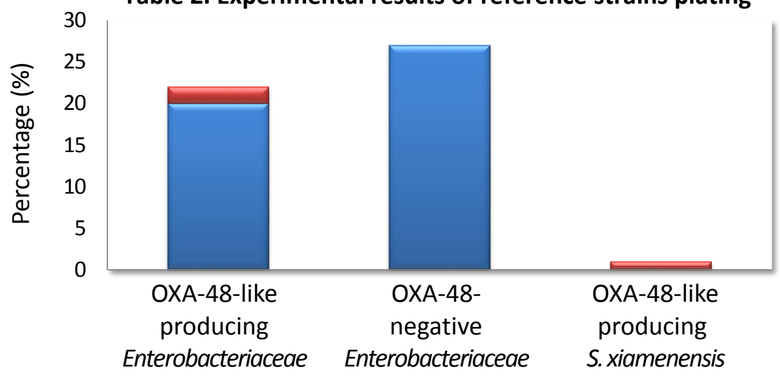

Table 3. Experimental results of LOD determination

	LOD [a]		
	Routine chromogenic plate	CDC	Chromatic OXA-48
E. coli	$<10^1$	6.7×10^2	6.7×10^4
K. pneumoniae	$<10^1$	8.3×10^1	$<10^1$
E. cloacae complex	$<10^1$	$<10^1$	1.3×10^5

[a] Expressed as CFU needed to observe growth in all the three replicates

Materials and methods

Phase I: 30 µl of liquid phase rectal swabs (n=150) obtained from Careggi University Hospital inpatients, have been streaked both on the "Chromatic OXA-48" plates and on chromogenic selective medium routinely used in the laboratory. Plates were incubated for 16-20 hours at 35±2°C, and colonies identified by MALDI-TOF. *Enterobacteriaceae* and non-fermenting Gram-negative bacteria have been analyzed by a multiplex-RT-PCR protocol able to detect $bla_{\text{KPC-type}}$, $bla_{\text{NDM-type}}$, $bla_{\text{VIM-type}}$, and $bla_{\text{OXA-48-like}}$ carbapenemase genes.

Phase II: "Chromatic OXA-48" plates have been inoculated with a 0.5 McFarland bacterial suspension of reference strains (n=50) by using a 10 µl loop. Isolates were selected with the aim to include all clinically relevant carbapenem resistance mechanisms (including the production of KPC, NDM, VIM, OXA-48 type enzymes and ESBL production with concomitant loss of outer membranes porins) as well as different MIC distribution to carbapenems **(Table 1** and **Table 2**). The collection included also an OXA-48-like producing *Shewanella xiamenensis.*

Phase III: Three OXA-48-producing clinical isolates were used for the determination of the Limit of Detection (LOD) (**Table 3**). For each isolate, ten fold serial dilutions (≈1×10^8 - 1×10^3 CFU/ml) were plated on product under evaluation and on the plates used for routine testing. 500 µl of the same cellular suspensions were used to inoculate 5 ml of TSB liquid medium, supplemented with a disk of meropenem (10 µg) (Bio-Rad, Italy). After 16-20 hours incubation at 35±2 °C, 100 µl were streaked to isolation on MacConkey agar plates according to the CDC protocol (reference method). Plates were read after 16-20 hours at 35±2 °C. All experiments were performed in triplicate.

Results

Phase I: direct testing of rectal swabs gave the expected results in 149/150 cases, and only in one case the growth of a carbapenem resistant *Acinetobacter baumannii* was observed. No OXA-48-producing strain of *Enterobacteriaceae* was detected.

Phase II: the Chromatic OXA-48 medium correctly detected 20/22 OXA-48 producers among the reference strains, with no false positives (**Table 1**).

Phase III: the LOD was 6.7×10^6, 1.3×10^7, and $<10^1$ CFU, for *E. coli*, *E. cloacae* and *K. pneumoniae*, respectively (**Table 3**).

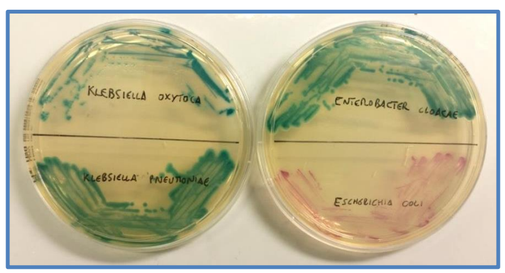

Figure 1. Examples of OXA-48-producing *Enterobacteriaceae* grown on Chromatic™ OXA-48 medium

Conclusions

The evaluated product showed a high specificity for OXA-48-producing *Enterobacteriaceae*.

The determination of the LOD resulted in a better sensitivity than the reference method for OXA-48-producing *K. pneumoniae,* whereas the sensitivity was lower for *E. coli* and *E. cloacae*.