Scope of the document. This document gives recommendations on laboratory methods for screening for colonisation with carbapenemase-producing *Enterobacteriaceae* (CPE). Indications for screening, the number and interval of screening samples and the consequences of a positive or negative result are outwith the scope of this document and should be determined by local or national infection control policies.

Background

Carbapenemases are beta-lactamases (enzymes) that hydrolyze carbapenems, i.e. any or all of ertapenem, meropenem, imipenem or doripenem. Carbapenemases are produced by several gram-negative species and the mechanism may be intrinsic (natural) or acquired, usually plasmid-mediated. This document focuses on *Enterobacteriaceae* with acquired carbapenemase-encoding genes, collectively referred to as carbapenemase-producing *Enterobacteriaceae* (CPE).

With regard to clinical infections and infection control, the most important host species are *Klebsiella pneumoniae*, *Escherichia coli* and *Enterobacter cloacae*, and the main acquired carbapenemases are KPC, OXA-48, NDM, VIM and IMP.

The level of carbapenem resistance varies and carbapenem MICs for CPE strains span from below the clinical susceptible breakpoints (yet above ECOFF) to high-level resistance. This makes screening strategies challenging, particularly with regards to finding a single method to detect all types of carbapenemases reliably and cost-effectively.

Decreased susceptibility to carbapenems in *Enterobacteriaceae* may also be due to non-transferable mechanisms such as ESBL or AmpC enzymes combined with changes in the permeability of the outer membrane of the bacterial cell wall. However, strains with non-transferable mechanisms are often considered less important from an infection control perspective than those producing acquired carbapenemases.

Screening

Sample type. Screening for CPE should always include a rectal swab or faecal specimen. Additional sites may be sampled, see national or local guidelines for recommendations.

Screening methods. Selective culture using antibiotic containing media (commercial or in-house) or antibiotic discs on non-antibiotic media are the most widely used methods of screening for CPE. Direct molecular methods may also be used, however the final decision on carriage should be based on isolation of a CPE strain. This also enables extended susceptibility testing (treatment options in case of infection) and typing (epidemiology).

Selective culture on antibiotic-containing chromogenic media is currently the most convenient method for CPE screening. Several commercially available chromogenic media have shown good performance in comparison to non-antibiotic agars with an applied carbapenem disc, or in-house agars containing imipenem (14, 15, 18). Studies describing the performance of various media are summarised in Table. When assessing these studies and choosing a method for CPE screening the following points should be taken into account:

- Strains with low carbapenem MICs may not grow on all commercial chromogenic media. In particular, detection of strains producing OXA-48 or OXA-48-like enzymes may be problematic, although some agars have been designed specifically for them. A combination of two chromogenic agars may thus be necessary to offer maximum sensitivity (6, 7, 13, 23). See Table for details.
- Using a prolonged incubation period (from 18-24 to 48 h) does not necessarily increase sensitivity when using chromogenic media, and may decrease specificity (8, 22).
- Enrichment in broth prior to plating on a chromogenic CPE media seems to increase sensitivity, especially for detection of strains producing OXA-48 (7, 8, 9). Broths used in the studies have been with (7, 9, 15) or without (8, 19) a selective carbapenem. A disadvantage of pre-enrichment is that an extra day is required to obtain results, but if combined with either direct culture on selective media or a molecular test, optimum speed and sensitivity can be achieved.
- When using selective media, a growth of faecal bacteria on simultaneously inoculated nonselective agar may help validate a negative screening result, whilst no growth of faecal bacteria may suggest the abscence of faecal material and an invalid sample.
- Studies are often performed in a single location where a single carbapenemase predominates, using media optimised for this specific enzyme. This media may therefore perform differently when required to detect a broad range of carbapenemases in a non-endemic setting.
- Some studies may seem to favor commercial media due to a suboptimal comparator method being used, such as in-house agars incorporating imipenem (which is not recommended by EUCAST for screening of CPE) or because inappropriate carbapenem disc zone sizes for screening were used (1).

Confirmatory tests on suspect CPE colonies

Identification to species level is necessary to exclude non- *Enterobacteriaceae* which may grow on the agars and to interpret the antimicrobial susceptibility patterns.

Decreased susceptibility to carbapenems should be confirmed by disc diffusion or a MIC-method. Screening cut-offs recommended by EUCAST are presented in the resistance mechanism document. Specificity of the screening can be improved by only testing strains that are resistant to piperacillin-tazobactam, and testing for susceptibility for temocillin in *Enterobacter* species.

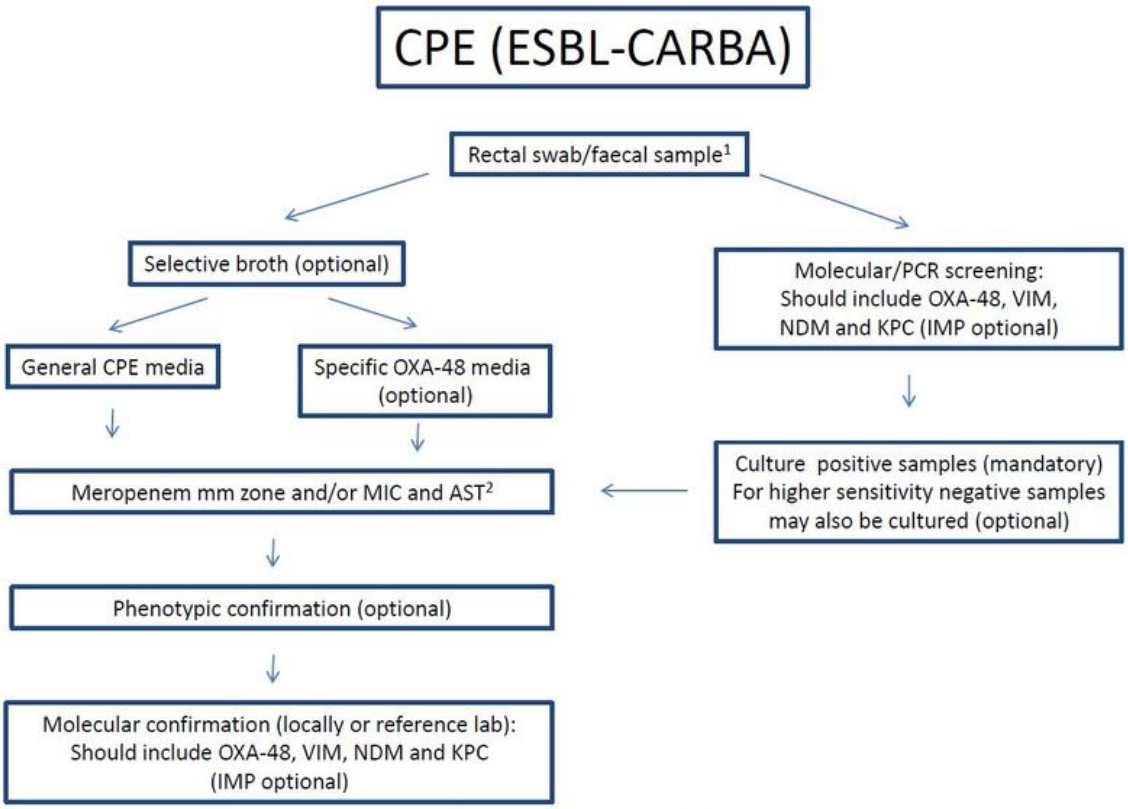
Some phenotypic confirmation tests, such as synergy tests and Carba NP tests are discussed in the above mentioned EUCAST resistance method document
More recently introduced tests include Blue Carba (17), carbapenem inactivation method (CIM,24), and a rapid immunochromatographic lateral-flow device (10). Phenotypic confirmation of carbapenemase production by Maldi-TOF is under commercial development and shows promising results (11). None of these phenotypic tests can replace molecular confirmation in Nordic settings, but an advantage is that in principle all types of carbapenemases can be detected in a short time frame.

Final confirmation of CPE is best done by molecular detection of a carbapenemase encoding gene. Molecular tests are also the only reliable means of detecting production of multiple carbapenemases in an isolate. Several commercial tests are available and validated in-house assays may also be used. A confirmatory test should at least cover the clinically most important genes: KPC, OXA-48, NDM and VIM (IMP is rarely found in Nordic countries). Choice of test will depend on preferred gene coverage, intended throughput, cost and ability to fit into local workflows.

Direct molecular screening

Molecular methods may also be used directly to detect CPE in clinical specimens. They have a shorter turnaround time and may have better sensitivity than culture-based methods (2, 12, 18, 19). However, studies have been undertaken in endemic settings and there are very limited data on the performance of direct molecular assays in low-prevalence settings with various CPE strains. The rarity of positive findings precludes appropriate validation of in-house molecular assays and only well-validated commercial assays should therefore be used for direct screening.

The most important limitation for direct molecular screening is that the range of detectable strains is limited to those targeted by the assay. Molecular methods will not identify producers of rare or new carbapenemases, neither do they provide information on the host species. Thus they should only be used in combination with culture-based methods and the final decision of carriage status should be based on isolation of a CPE strain.



1 CPE-screening must include a rectal swab or faecal sample. Other relevant sites may also be sampled.
2 According to EUCAST/NordicAST recommendations.

Table. **Studies on selective agars for detection of carbapenemases in *Enterobacteriaceae*** (abbreviations: MacC, MacConkey; w/o, without; w., with)

References	Media	Sensitivity (%)	Specificity (%)	Material tested	Comments
Henrichs et al 2016 (8)	ChromID CARBA	29	100	730 rectal swabs: 17 OXA-48-like, 2 VIM	
	Brilliance CRE	79	97		
	MacC broth w/o antibiotics and ChromID CARBA	42	NA		
	MacC broth w/o antibiotics and ChromID CARBA	95	NA		
Zarakolou et al 2015 (23)	TSB w. meropenem (2mg/L)	57.6	95.2	302 rectal swabs, 33 OXA-48	
	ChromID OXA-48 + ChromID CARBA	90.9	98.5		
	ChromID OXA-48 + TSB w. meropenem (2mg/L)	90.9	94.8		
	ChromID CARBA+ TSB w. meropenem (2mg/L)	75.8	94.4		
	CHromID CARBA	57.6	98.9		
	ChromID OXA-48	75.8	99.3		
Glaser et al 2015 (9)	TSB w. meropenem (2mg/L) + MacConkey w. disc	85	-	238 rectal swabs, 35 CREs Probably all KPCs	Not all specimens tested with all three methods
	ChromID CARBA	53	-		
	TSB w. meropenem (2mg/L) + MacConkey w. disc	95	-		
Vasoo et al 2014 (20)	Spectra CRE	97.8	86.4	150 perirectal swabs, 47 KPC (49 strains)	
	Chromagar KPC	76.6	75.7		
	MacC w. ertapenem disc	83.0	73.8		
Girlich et al 2014 (7)	ChromID ESBL	90 (w/o enrichment) 100 (w. enrichment)	29.9	77 rectal swabs, 10 OXA-48	BHI enrichment broth w. 0.25 mg/L ertapenem
	Brilliance CRE	80 (w/o enrichment) 100 (w. enrichment)	71.6		
	SUPERCARBA	80 (w/o enrichment) 100 (w. enrichment)	52.2		
Papadimitriou-Olivgeris et al 2014 (15)	ChromID CARBA	96.5	91.2	177 rectal swabs 88 KPC, 1 VIM	
	MacConkey w. imipenem disc	89.5	31.9		
	TSB w. ertapenem (2 mg/L)	98.8	80.2		
Girlich et al 2013 (5)	SUPERCARBA	96.5	60.7	142 isolates 43 OXA-48, 20 KPC, 18 VIM, 17 IMP, 16 NDM	Study used bacterial suspensions (not clinical samples) for evaluation of CPE detection
	Brilliance CRE	76.3	57.1		
	CHROMagar KPC	43	67.8		
References	**Media**	**Sensitivity (%)**	**Specificity (%)**	**Material tested**	**Comments**
Girlich et al 2013 (6)	SUPERCARBA	96.1	52.5	117 isolates 57 OXA-48-like, 10 KPC, 10 MBL	Study used bacterial suspensions (not clinical samples) for evaluation of CPE detection
	ChromID OXA-48	70.1	100 (for OXA-48)		
	ChromID CARBA	40.3	67.5		

	ChromID OXA-48 & CARBA	94.8	67.5		
Day et al 2013 (3)	**Brilliance CRE**	57	12	175 stool samples, 37 NDM	
	ChromID CARBA	94.5	87.5		
Day et al 2013 (4)	**Brilliance CRE**	54	23	152 stool samples, 16 NDM	
	ChromID CARBA	85	85		
Wilkinson et al 2012 (22)	**Brilliance CRE**	78	66	200 isolates, 88 NDM, 9 IMP, 12 KPC, 15 OXA-48, 6 VIM	Study used bacterial suspensions (not clinical samples) for evaluation of CPE detection
	ChromID CARBA	91	89		
	ChromID ESBL	96	19		
	Colorex KPC	56	77		
	TSB w. ertapenem (2 mg/L)	78	69		
	TSB w. meropenem (2 mg/L)	47	79		
Vrioni et al 2012 (21)	**TSB w. ertapenem (2 mg/L)**	89.1	86.4	200 rectal swabs, 63 KPC, 29 VIM	
	BHI w. ertapenem (1 mg/L), ChromID ESBL	92.4	93.3		
	ChromID ESBL	92.4	84.7		
	ChromID CARBA	92.4	96.9		
	MacConkey w. meropenem disk	89.1	85.2		
Singh et al 2012 (19)	**ChromID ESBL**	77.3	100	95 rectal swabs, 66 KPC	PCR and subculture from enrichment broth on VACC
	VACC (vanco, ampho B, cefta, clinda)	77.3	100		
	TSB (6 mg/L ceftazidime)	97	-		
	MacConkey +ChromID CARBA	87.5	-		
Adler et al 2011 (1)	**CHROMagar KPC**	84.9	88.7	139 rectal swabs, 32 KPC	The carbapenem disc zone diameters used differ from those recommended by EUCAST
	MacConkey w. imipenem (1 mg/L)	84.9	94.3		
	MacConkey w. carbapenem discs	75.8	89.6		
Perry et al 2011 (16)	**Colorex KPC**	64.1	-	200 rectal specimens, 64 NDM	Samples were locally cultured on MacC, frozen mixed growth on chromagars
	ChromID CARBA	87.5	-		
Samra et al 2008 (18)	**CHROMagar KPC**	100	98.4	120 rectal swabs, 41 KPC	
	MacConkey w. carbapenem discs	92.7	95.9		

References

Recommendations:

AFA K-RES 2015-11-26. Litteraturgjennomgang og vurdering av mulige strategier ved bruk av selektive kromogene medier for screening for bærerskap av multiresistente (ESBL-holdige) Gram-negative stavbakterier https://unn.no/Documents/Kompetansetjenester.%20-sentre%20og%20fagråd/AFA%20-%20arbeidsgruppen%20for%20antibiotikaspørsmål/Metoder/20151126%20Screening%20ESBL%20AFA%20KRES%20(2).pdf

DanRes-M.Screening ESBL.Undersøgelse for Carbapenemase Producerende Organismer (CPO) bærertilstand - en metodevejledning http://dskm.dk/onewebmedia/Unders%C3%B8gelse%20for%20Carbapenemase%20Producerende%20Organismer%20(CPO)%20b%C3%A6rertilstand%20-%20en%20metodevejledning.pdf

EUCAST. EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Resistance_mechanisms/EUCAST_detection_of_resistance_mechanisms_v1.0_20131211.pdf

Public Health England. Screening and Detection of Bacteria with Carbapenem-Hydrolysing β-lactamases (Carbapenemases). UK Standards for Microbiology Investigations. B 60 Issue. https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories

Kolho, Lyytikäinen, Jalava. Ohje moniresistenttien mikrobien tartunnantorjunnasta. THL-Ohjaus 22/2017
http://urn.fi/URN:ISBN:978-952-302-943-9

Original publications (*presented in Table)

1. *Adler A, NavonVenezia S, MoranGilad J, Marcos E, Schwartz D, Carmeli Y. Laboratory and clinical evaluation of screening agar plates for detection of carbapenemresistant Enterobacteriaceae from surveillance rectal swabs. J Clin Microbiol. 2011;49(6):223942.
2. Avlami A, Bekris S, Ganteris G, Kraniotaki E, Malamou-Lada E, Orfanidou M, et al. Detection of metallo-beta-lactamase genes in clinical specimens by a commercial multiplex PCR system. J Microbiol Methods 2010;83:185-7.
3. *Day KM, Ali S, Mirza IA, Sidjabat HE, Silvey A, Lanyon CV, Cummings SP, Abbasi SA, Raza MW, Paterson DL, Perry JD. Prevalence and molecular characterization of Enterobacteriaceae producing NDM-1 carbapenemase at a military hospital in Pakistan and evaluation of two chromogenic media.Diagn Microbiol Infect Dis. 2013;75(2):187-91.
4. *Day KM, Salman M, Kazi B, Sidjabat HE, Silvey A, Lanyon CV, Cummings SP, Ali MN, Raza MW, Paterson DL, Perry JD. Prevalence of NDM-1 carbapenemase in patients with diarrhoea in Pakistan and evaluation of two chromogenic culture media. J Appl Microbiol. 2013;114(6):1810-6.
5. *Girlich D, Poirel L, Nordmann P. Comparison of the SUPERCARBA, CHROMagar KPC, and Brilliance CRE screening media for detection of Enterobacteriaceae with reduced susceptibility to carbapenems. Diagn Microbiol Infect Dis 2013;75:214-7.
6. *Girlich D, Anglade C, Zambardi G, Nordmann P. Comparative evaluation of a novel chromogenic medium (chromID OXA-48) for detection of OXA-48 producing Enterobacteriaceae. Diagn Microbiol Infect Dis 2013;77(4):296-300.
7. *Girlich D, Bouihat N, Poirel L, Benouda A, Nordmann P. High rate of faecal carriage of extended-spectrum β-lactamase and OXA-48 carbapenemase-producing Enterobacteriaceae at a University hospital in MoroccoClin Microbiol Infect. 2014 Apr;20(4):350-4.
8. Glaser L, Andreacchio K, Lyons M, Alby K. Improved surveillance for carbapenem-resistant Enterobacteriaceae using chromogenic media with a broth enrichmentDiagn Microbiol Infect Dis. 2015 Aug;82(4):284-5.
9. *Heinrichs A, Nonhoff C, Roisin S, De Mendonça R, Adam AS, Dodémont M, Denis O. Comparison of two chromogenic media and enrichment broth for the detection of carbapenemase-producing Enterobacteriaceae on screening rectal swabs from hospitalized patients J Med Microbiol. 2016;65(5):438-41.
10. Meunier D, Vickers A, Pike R, Hill RL, Woodford N, Hopkins KL. Evaluation of the K-SeT R.E.S.I.S.T. immunochromatographic assay for the rapid detection of KPC and OXA-48-like carbapenemases. J Antimicrob Chemother. 2016 Aug;71(8):2357-9.
11. Monteferrante CG, Sultan S, Ten Kate MT, Dekker LJ, Sparbier K, Peer M, Kostzrewa M, Luider TM, Goessens WH, Burgers PC. Evaluation of different pretreatment protocols to detect accurately clinical carbapenemase-producing Enterobacteriaceae by MALDI-TOF. J Antimicrob Chemother. 2016 Oct;71(10):2856-67
12. Naas T, Cotellon G, Ergani A, Nordmann P. Real-time PCR for detection of blaOXA-48 genes from stools. J Antimicrob Chemother 2013;68:101-4.
13. Nordmann P, Girlich D, Poirel L. Detection of carbapenemase producers in Enterobacteriaceae by use of a novel screening medium. *Journal of Clinical Microbiology* 2012;**50**:2761-6.
14. Panagea T, Galani I, Souli M, Adamou P, Antoniadou A, Giamarellou H. Evaluation of CHROMagar™ KPC for the detection of carbapenemaseproducing Enterobacteriaceae in rectal surveillance cultures. Int J Antimicrob Agents. 2011;37(2):1248.
15. *PapadimitriouOlivgeris M, Bartzavali C, Christofidou M, Bereksi N, Hey J, Zambardi G, Spiliopoulou I. Performance of chromID® CARBA medium for carbapenemasesproducing Enterobacteriaceae detection during rectal screening. Eur J Clin Microbiol Infect Dis. 2014;33(1):3540.
16. *Perry JD, Naqvi SH, Mirza IA, Alizai SA, Hussain A, Ghirardi S et al. Prevalence of faecal carriage of Enterobacteriaceae with NDM-1 carbapenemase at military hospitals in Pakistan, and evaluation of two chromogenic media. J Antimicrob Chemother 2011;66:2288-94.
17. Pires J, Novais A, Peixe L. Blue-carba, an easy biochemical test for detection of diverse carbapenemase producers directly from bacterial cultures. J Clin Microbiol. 2013;51(12):4281-3.
18. *Samra Z, Bahar J, MadarShapiro L, Aziz N, Israel S, Bishara J. Evaluation of CHROMagar KPC for Rapid Detection of CarbapenemResistant Enterobacteriaceae. Journal of Clinical Microbiology. 2008;46(9):3110-3111.
19. *Singh K, Mangold KA, Wyant K, Schora DM, Voss B, Kaul KL, et al. Rectal screening for Klebsiella pneumoniae carbapenemases: comparison of real-time PCR and culture using two selective screening agar plates. J Clin Microbiol 2012;50:2596-600.
20. Vasoo S, Lolans K, Li H, Prabaker K, Hayden MK. Comparison of the CHROMagar KPC, Remel Spectra CRE, and a direct ertapenem disk method for the detection of KPC-producing Enterobacteriaceae from perirectal swabs. Diagn Microbiol Infect Dis 2014;78:356-9.
21. *Vrioni G, Daniil I, Voulgari E, Ranellou K, Koumaki V, Ghirardi S, Kimouli M, Zambardi G, Tsakris A. Comparative evaluation of a prototype chromogenic medium (ChromID CARBA) for detecting carbapenemaseproducing Enterobacteriaceae in surveillance rectal swabs. J Clin Microbiol. 2012 Jun;50(6):18416.
22. *Wilkinson KM, Winstanley TG, Lanyon C, Cummings SP, Raza MW, Perry JD. Comparison of four chromogenic culture media for carbapenemaseproducing Enterobacteriaceae. J Clin Microbiol. 2012 Sep;50(9):31024
23. *Zarakolu P, Day KM, Sidjabat HE, Kamolvit W, Lanyon CV, Cummings SP, Paterson DL, Akova M, Perry JD. Evaluation of a new chromogenic medium, chromID OXA-48, for recovery of carbapenemase-producing Enterobacteriaceae from patients at a university hospital in Turkey. Eur J Clin Microbiol Infect Dis. 2015 Mar;34(3):519-25.
24. van der Zwaluw K, de Haan A, Pluister GN, Bootsma HJ, de Neeling AJ, Schouls LM. The carbapenem inactivation method (CIM), a simple and low-cost alternative for the Carba NP test to assess phenotypic carbapenemase activity in gram-negative rods. PLoS One. 2015 Mar 23;10(3):e0123690.

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Changes

Version	Changes
2017-01-30	New document
2017-30-11	screening cutoff according to EUCAST v 2.0 document