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Warning

Carbapenemase OXA-244 in *E. coli* in Switzerland

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Carbapenemase producers in Enterobacterales are increasingly identified in Switzerland not only in *Klebsiella pneumoniae* but also in *Escherichia coli*. Whereas the main determinants in *K. pneumoniae* remain of the KPC type, the NDM type and OXA-48 types enzymes are mostly identified in *E. coli*.

OXA-48-type carbapenemases possess a weaker carbapenemase activity than KPC or NDM carbapenemases (Nordmann et al. 2019) A series of point mutations variant of OXA-48 have been reported. The OXA-181, OXA-232, OXA-204, OXA-162, and OXA-244, in that order are the most common enzymes that are identified among the OXA-48 group (Poirel *et al.* 2012). Not all the OXA-48 variants possess the same carbapenemase activity, such as OXA-244 which has a lower carbapenemase activity compared to OXA-48 (Oteo *et al.*, 2013; Potron *et al.*, 2016). Some of those OXA-48 variants are even devoid of any carbapenemase activity such as OXA-163. Isolates producing OXA-48 derivatives are usually resistant to temocillin that is a uncommon property not shared with those producing the two other carbapenemase groups (KPC/NDM) (Huang *et al.*, 2014.).

Although we are observing a globalization of strains possessing carbapenemase genes, OXA-48-like producers are spread in particular in North Africa, Africa, Middle East, Africa and India (Nordmann *et al.*, 2019). In Europe they are increasingly reported as the main carbapenemases at least in France and Germany.

Many methods are used to identify OXA-48 like producers including phenotypic susceptibility testing, selective culture media, immunochromatographic assays, specific PCRs and sequenced-based molecular tests (Decousser *et al.*, 2017). All techniques display variable degrees of sensitivity and specificity.

Warning; We would like to draw your attention on the increase identification of OXA-244 producers in *E. coli* from different parts of Switzerland (Aarau, Bern, Basel, Fribourg, Lausanne, Luzern, Sion) since the beginning of 2019. Those strains are either responsible of infections (urinary tract infections) or are just colonizer of the gut flora. Similar observation has been made very recently at least in France, Austria, and Germany (Hoyos-Mallecot *et al.*, 2017) .

OXA-244 is a point-mutant derivative of OXA-48 with Arg214Gln substitution that was identified first in Malaga in Spain (Oteo *et al.*, 2013). Then it was identified from different part of the world including Russia, Indonesia, The Netherlands, Germany, UK, Egypt and

France, mostly in *E. coli* (Potron *et al*, 2016; Hoyos-Mallecot *et al*, 2017) A national survey performed in the UK identified OXA-244 producers in 10% of OXA-48-like producers in *E. coli* from 2007 to 2014 (Findlay *et al*, 2017). It has a weaker carbapenemase activity as compared to OXA-48 (Potron *et al*, 2017). It hydrolyzes temocillin less than OXA-48 does, temocillin resistance being a common property of OXA-48 derivatives (Potron *et al*, 2017; Huang *et al*, 2014). Its gene is located on a Tn1999 transposon (as for the OXA-48 encoding gene) that explains in part its spread from plasmid to plasmid and from plasmid to chromosome et vice-versa (Hoyos-Mallecot *et al*, 2017).

The sixteen OXA-244 producers in *E. coli* which identification has been confirmed at the NARA since the beginning of the year showed variable degrees of susceptibility/resistance to carbapenems and to expanded-spectrum cephalosporins.

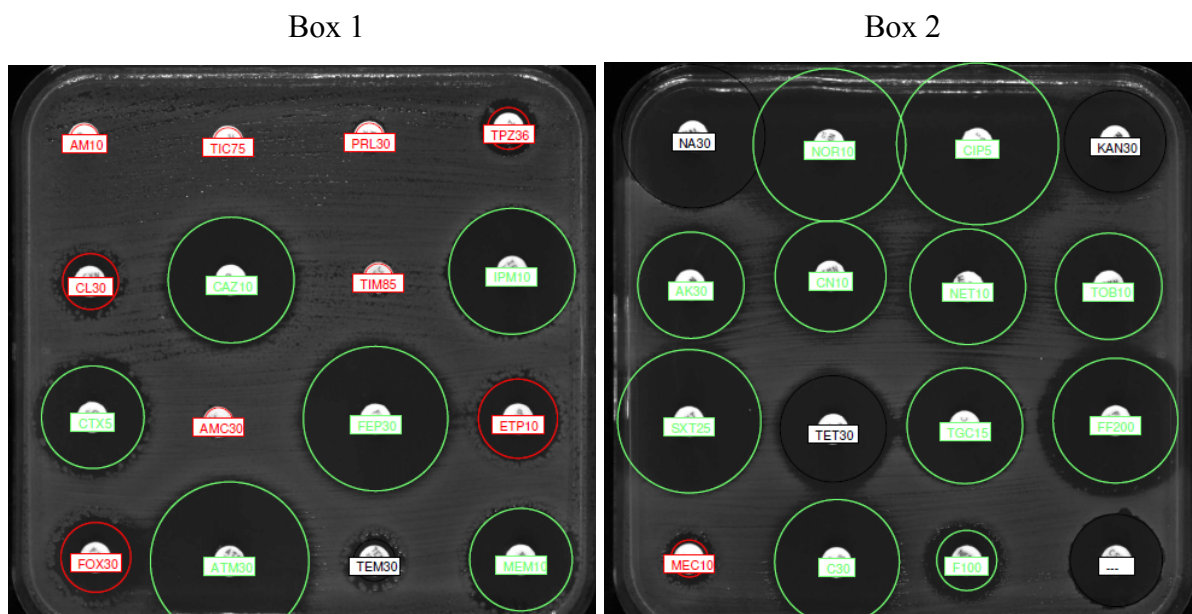
	MIC range (mg/L)	Geometric mean (mg/L)
Ertapenem	0.125 - >32	5
Meropenem	0.047 - 1.5	0.4
Imipenem	0.19 – 1	0.5
Ceftazidime	0.125 – 16	3.5
Ceftazidime-Avibactam	0.032 – 0.75	0.2

Variations in MICs of carbapenems for those strains may be related to the plasmid or chromosomal location of the *bla*_{OXA-244} gene, as described (Potron *et al*, 2016; Hoyes-Mallecot *et al*, 2017). All strains remained susceptible to the combination of ceftazidime/avibactam.

Those strains do neither belong to a single ST type nor to a single clone. However a major ST-38 clone is observed, as previously published (Hoyos-Mallecot *et al*, 2017; Potron *et al*, 2016) This is a logical finding since most of the OXA-48 producers in *E. coli* are also of the same ST-38 type (Gauthier *et al*, 2018; Izdebski *et al*, 2018; Zurfluh *et al*, 2015).

Most but not all strains co-express an ESBL that is CTX-M-14 or CTX-M-27 (a point mutant variant of CTX-M-14) and not the very common ESBL CTX-M-15. Co-resistances to other families of antibiotics vary from strain to strain (tetracyclines, sulfonamides, aminoglycosides, fluoroquinolones).

See below an example of an OXA-244 producer in *E. coli*.



Box 1 : AM, Ampicillin (10 µg) ; TIC, Ticarcillin (75 µg) ; PRL, Piperacillin (30 µg) ; TPZ, Piperacillin + Tazobactam (30/6 µg) ; CL, Cefalexin (30 µg) ; CAZ, Ceftazidime (10 µg) ; TIM, Ticarcillin + clavulanic acid (75/10 µg) ; IPM, Imipenem (10 µg) ; CTX, Cefotaxime (5 µg) ; AMC, Amoxicillin + clavulanic acid (20/10 µg) ; FEP, Cefepime (30 µg) ; ETP, Ertapenem (10 µg) ; FOX, Cefoxitin (30 µg) ; ATM, Aztréonam (30 µg) ; TEM, Temocillin (30 µg) ; MEM, Meropenem (10 µg).

Box 2 : NA, nalidixic acid (30 µg) ; NOR, norfloxacin (10 µg) ; CIP, ciprofloxacin (5 µg) ; KAN, Kanamycin (30 µg) ; AK, Amikacin (30 µg) ; GMN, Gentamicin (10 µg) ; NET, Netilmicin (10 µg) ; TOB, Tobramycin (10 µg) ; SXT, Trimethoprim + Sulfamethoxazole (1,25/23,75 µg) ; TET, Tetracyclin (30 µg) ; TGC, Tigecyclin (15 µg) ; FF, Fosfomycin (200 µg) ; MEC, Mecillinam (10 µg) ; C, Chloramphenicol (30 µg) ; F, Nitrofurantoin (100 µg).

Our recommendation to detect the OXA-244 strains are the followings.

1- Susceptibility testing

The 2019 updated EUCAST breakpoints for carbapenems are as followed:

Carbapenems	MIC breakpoints (mg/L)		Disk content (µg)	Zone diameter breakpoints (mm)	
	S ≤	R >		S ≥	R <
<u>Ertapenem</u>	0.5	0.5	10	25	25
<u>Imipenem</u>	2	4	10	22	17
<u>Meropenem</u>	2	8	10	22	16

However, the recommended criteria for detection of carbapenemase production are different and are as follows;

Table 3. Recommended screening criteria for detection of carbapenemase-producing Enterobacterales in 2018 CLSI and EUCAST guidelines.

Susceptibility method	Antimicrobial agent	MIC (µg/ml) or disk diameter (mm)	
		CLSI	EUCAST
Broth microdilution	Meropenem	≥2	>0.125
	Ertapenem	≥2	>0.125
	Imipenem	≥1	-
Disk diffusion	Meropenem	≤20	<28 ^a
	Ertapenem	≤19	<25
	Imipenem	≤20	-

2 – Identification of biochemical activity

A biochemical detection of carbapenemases may be based on the rapid detection of imipenem hydrolysis (Rapid Carba NP test: Rapidec Carba NP test (bioMérieux, Geneva) (CLSI/EUCAST guidelines, 2019) (Decousser *et al*, 2017; Mancini *et al*, 2017; Nordmann *et al*, 2012). Results are obtained mostly in 30 min-1 h. Use of an adequate inoculum (1 öse of 10 µl) is critical to detect carbapenemase activity. Another biochemical technique is the β-Carba test based on the hydrolysis of a chromogenic carbapenem (Bio-Rad, Cressier) (Decousser *et al*, 2017).

We have compared those two techniques for those OXA-244-producing *E. coli* isolates and results are as follows :

Carba NP (in-house) : negative (detection 0/16)

Rapidec Carba NP test : negative (detection 0/16)

B-Carba test: positive for 10/16 strains

This result is related to the weak carbapenemase activity of OXA-244.

3- Immunological detection

Lateral flow techniques have been commercialized for detecting the most common carbapenemases of the KPC, NDM, VIM, OXA-48 types. Two companies, namely Coris Bioconcept (Gembloux, Belgium) and NG Biotech (Guipry, France) have marketed recently those lateral flow techniques (Dortet *et al*, 2016; Kieffer *et al*, 2019). The test developed by NG Biotech additionally detects IMP producers. Both tests are rapid (15 min), sensitive and specific. We have tested the NG Biotech test that perfectly detected this OXA-48 derivative in 16/16 strains. This result is explained by the fact that OXA-244 is just a point-mutant derivative of OXA-48 and therefore well-detected with monoclonal antibodies directed to OXA-48.

4- Screening of patients possibly colonized with OXA-244 producers

Several screening media have been developed for screening carbapenemases producers in Enterobacterales from stools. Of note, not all of those media may screen for all types of carbapenemase producers.

The ChromID[®] Carba SMART (bioMérieux) and mSuperCARBA (CHROMagar) can screen theoretically any type of carbapenemase producer (Girlich *et al*, 2013, Nordmann *et al*, 2012; Viau *et al*, 2016). The Carba SMART is a biplate that contains on one side a carbapenem molecule (possibly imipenem) and in the other side temocillin, taking into account that most OXA-48-producers are temocillin resistant. The mSuperCarba contains ertapenem.

Several concentrations of each OXA-244 have been tested (10^8 , 10^6 , 10^4 , 10^2 CFU/ml) have been tested on ChromID[®] Carba SMART (bioMérieux) and mSuperCARBA (CHROMagar).

At the highest inoculum (10^8 CFU/mL) (higher than the concentration observed in clinical stools, 10^1 , 10^4 CFU/mL), 2/16 of OXA-244 producers grew on ChromID[®]

Carba SMART (on the temocillin-containing part of the bi-plate). At lower inocula, the Carba SMART failed to detect the OXA-244 producers. This result is explained by the fact that high-level resistance to temocillin is not observed for all the OXA-244 strains, most of them just showing decreased susceptibility to carbapenems.

At the highest inoculum (10^8 CFU/mL), 15/16 OXA-244 producers are detected on the mSuperCARBA medium. At lower inocula, the results are as follows; 10^6 CFU/ml, 13/16 strains; 10^4 CFU/ml, 9/16 strains; 10^2 CFU/ml, 8/16 strains. This result is explained by the fact that the screening molecule here is ertapenem at a relatively low concentration.

The recommendation is therefore to use the mSuperCarba medium which shows the best sensitivity of detection.

Two situations may be observed;

- A non-outbreak situation ; direct plating of rectal swabs or stools on mSuperCarba medium
- An outbreak situation:
 - Direct plating on mSuperCarba and/or use of molecular techniques for detecting OXA-48-like producers (XpertCarba-R, (Cepheid) Amplidiag CarbaR+VRE, Amplidiag CarbaR+MCR (Mobidiag) Check Direct CPE on BD MAXTM (Check-Points) eazyplexSuperBug CRE (OptiGene) Carbaplex IVD PCR (Brucker), CRE ELITe MGB Kit (ELITech), BioFire Multiplex Film Array BCDI2 Panel (bioMérieux), Revogene (GenePOC)... (Decousser *et al*, 2017; Findlay *et al*, 2015; Naas *et al*, 2013; Oueslati *et al*, 2018)
 - Concomitant cultures of the rectal or stool samples in an enrichment broth containing ertapenem 0.1 mg/L to improve the sensitivity of the detection based on the results of a study we performed recently (Sadek *et al*, 2019)

5- Treatment

Although carbapenem susceptibility may vary from one strain to the other, if a β -lactam is chosen, we are reluctant to recommend the use of any carbapenem for treating infections due to the OXA-244 producers. Those OXA-244 producers remain susceptible to the combination ceftazidime-avibactam. In addition, it should be underlined many of those strains do not coproduce an ESBL and remain susceptible to aminoglycosides and fluoroquinolones. Therefore many therapeutic alternatives remain available for treating such infected patients.

6- References

- Decousser JW, Poirel L, Nordmann P. 2017. Recent advances in biochemical and molecular diagnostics for the rapid detection of antibiotic-resistant Enterobacteriaceae ; a focus on β -lactam resistance. *Exp Rev Mol Diagn.* 17:327-350.

- Dortet L, Jousset A, Sainte-Rose V, Cuzon G, Naas T. 2016. Prospective evaluation of the OXA-48 K-SeT assay, an immunochromatographic test for the rapid detection of OXA-48-type carbapenemases. *J Antimicrob Chemother.* 71:1834-1840.
- Findlay J, Hopkins KL, Loy R, Doumith M, Meunier D, Hill R, Pike R, Mustafa N, Livermore DM, Woodford N. 2017. OXA-48-like carbapenemases in the UK: an 1483 analysis of isolates and cases from 2007 to 2014. *J Antimicrob Chemother.* 72:1340-1349.
- Findlay J, Hopkins KL, Meunier D, Woodford N. 2015. Evaluation of three commercial assays for rapid detection of genes encoding clinically relevant carbapenemases in cultured bacteria. *J Antimicrob Chemother.* 70:1338-1342.
- Gauthier L, Dortet L, Cotellon G, Creton E, Cuzon G, Ponties V, Bonnin RA, Naas T. 2018. Diversity of carbapenemase-producing *Escherichia coli* isolates in France in 2012-2013. *J Antimicrob Chemother.* doi: 10.1128/AAC.00266-18.
- Girlich D, Anglade C, Zambardi G, Nordmann P. 2013. Comparative evaluation of a novel chromogenic medium (chromID OXA-48) for detection of OXA-48 producing Enterobacteriaceae. *Diagn Microbiol Infect Dis.* 77:296-300.
- Hoyos-Mallecot Y, Naas T, Bonnin RA, Patino R, Glaser P; Fortineau N, Dortet L. 2017. OXA-244-producing *Escherichia coli* isolate, a challenge for clinical microbiology laboratories. *Antimicrob Agents Chemother.* <https://doi.org/10.1128/AAC.00818-17>.
- Huang TD, Poirel L, Bogaerts P, Berhin C, Nordmann P, Glupczynski Y. 2014. Temocillin and piperacillin/tazobactam resistance by disc diffusion as antimicrobial surrogate markers for the detection of carbapenemase-producing Enterobacteriaceae in geographical areas with a high prevalence of OXA-48 producers. *J Antimicrob Chemother.* 68:445-55.
- Izdebski R, Baraniak A, Zabicka D, Machulska M, Ubanowicz P, Fiett J, Lieterack E, Bojarska K, Kozinska A, Zieniuk B, Hriyniewicz W, Gniadkowski M, OXA-49 PL group. 2018. Enterobacteriaceae producing OXA-48-like carbapenemases in Poland, 2013-January 2017. *J Antimicrob Chemother* 73:620-625.
- Kieffer N, Poirel L, Nordmann P. 2019. Rapid immunochromatography-based detection of carbapenemase producers. *Infection* 49:673-675.
- Mancini S, Kieffer N, Poirel L, Nordmann P. 2017. Evaluation of the Rapidec Carba NP and β -Carba tests for rapid identification of carbapenemase producing – Enterobacteriaceae. *Diag Microb Infect Dis.* 88:293-297.
- Naas T, Cotellon G, Ergani A, Nordmann P. 2013. Real-time PCR for detection of blaOXA-48 genes from stools. *J Antimicrob Chemother.* 68:101-104.
- Nordmann P, Girlich D, Poirel L. 2012. Detection of carbapenemase producers in Enterobacteriaceae by use of a novel screening medium. *J Clin Microbiol.* 50:2761-2766.
- Nordmann P, Poirel L, Dortet L. 2012. Rapid detection of carbapenemase-producing Enterobacteriaceae. *Emerg Infect Dis.* 18:1503-1507.
- Nordmann P, Poirel L. 2019. Epidemiology and diagnostic of carbapenem resistance in Gram-negative bacteria. *Clin Infect Dis.*, in press.
- Oteo J, Hernandez JM, Espasa M, Fleites A, Saez D, Bautista V, Perez-Vazquez M, Fernandez-Garcia MD, Delgado-Iribarren A, Sanchez-Romero I, Garcia-Picazo L, Miguel MD, Solis S, Aznar E, Trujillo G, Mediavilla C, Fontanals D, Rojo S, Vindel A, Campos J. 2013. Emergence of OXA-48-producing *Klebsiella pneumoniae* and the novel carbapenemases OXA-244 and OXA-245 in Spain. *J Antimicrob Chemother.* :317-321.

- Oueslati S, Girlich D, Dortet L, Naas T. 2018. Evaluation of the Amplidiag CarbaR+VRE kit for the accurate detection of carbapenemase-producing bacteria. *J Clin Microbiol.* 56 doi: 10.1128/JCM.01092-17
- Poirel L, Potron A, Nordmann P. 2012. OXA-48, the phantom menace. *J Antimicrob Chemother.* 67:1597-1606.
- Potron A, Poirel L, Dortet L, Nordmann. 2016. Characterisation of OXA-244, a chromosomally-encoded OXA-48-like β -lactamase from *Escherichia coli*. *Int J Antimicrob Chemother.* 47:101-106.
- Sadek M, Poirel L, Nordmann P. 2019. Optimal detection of extended-spectrum β -lactamase producers, carbapenem producers, polymyxin-resistant Enterobacterales, and vancomycin-resistant enterococci from stools. *Diag Microbiol Infect Dis.*, in press
- Viau R, Frank KM, Jacobs MR, Wilson B, Wilson B, Kaye K, Donskey CJ, Perez F, Endimiani F, Bonomo RA. 2016. Intestinal carriage of carbapenemase-producing organisms :current status of surveillance. *Clin Microbiol Rev.* 29:1-27.
- Zurfluh K, Nüesch Inderbinden MT, Poirel L, Nordmann P, Hächler H, Stephan R. 2015. Emergence of *Escherichia coli* OXA-48 producing β -lactamase in the community of Switzerland. *Antimicrob Resist Infect Control.* doi: 10.1186/s13756-015-0051-x