European Journal of Clinical Microbiology & Infectious Diseases
https://doi.org/10.1007/s10096-024-05007-2

BRIEF REPORT

Early detection of OXA-48 producing *Klebsiella pneumoniae* with the use of rapid antibiotic susceptibility testing

Emilie De Muynck[1] · Stien Vandendriessche[1] · Anne-Sophie Messiaen[1] · Kristen Decommer[2] · Diana Huis in 't Veld[2] · Jan J. De Waele[3,4] · Jerina Boelens[1,5]

Received: 11 September 2024 / Accepted: 27 November 2024

Abstract

Rapid antimicrobial susceptibility testing of positive blood cultures can enhance antimicrobial stewardship and patient outcomes. We present a case where OXA-48-producing *Klebsiella pneumoniae* with low-level carbapenem resistance was suspected 6 h after blood-culture positivity, based on ASTar system (Q-Linea, Sweden) results. OXA-48 carbapenemase presence was confirmed by the OXA-48 K-SeT lateral flow assay (Coris, Belgium) on a short-term subculture. This led to timely therapeutic adjustments. Following this case, we validated the OXA-48 K-SeT and Xpert Carba-R assay (Cepheid, USA) on short-term incubated (6 h) subcultures of positive blood cultures, demonstrating that OXA-48 K-SeT and Xpert Carba-R respectively accurately identified OXA-48 and various (OXA-48, VIM, NDM, KPC, IMP) carbapenemase-producing *Enterobacterales* (CPE) types. Both assays prove valuable for rapid CPE detection in clinical settings.

Introduction

Rapid antimicrobial susceptibility testing (RAST) of blood cultures may assist in enhancing antimicrobial stewardship (AMS), facilitate infection prevention and control (IPC) measures and improve patient outcomes. To achieve this, it is crucial to be able to detect carbapenemase-producing *Enterobacterales* (CPE) with low-level resistance to carbapenems. According to EUCAST guidelines, screening for CPE is recommended when the minimal inhibitory concentration (MIC) for meropenem or ertapenem exceeds 0.125 mg/L [1]. However, detection methods such as lateral flow assays (LFA), colorimetric assays or molecular assays are often only validated on bacterial colonies which have been cultivated for 18 to 24 h and which may thus not yet be available at the timing of RAST results. Furthermore, temocillin is proposed as a phenotypic marker for OXA-48(-like) CPE, but susceptibility testing for this antibiotic is not included in available AST platforms, as temocillin is not widely used. We report a case where CPE-type OXA-48 with low level carbapenem resistance was identified 6 h after positivity of the blood culture using the RAST ASTar-system (Q-linea, Uppsala, Sweden) in combination with the performance of an LFA (OXA-48 *K*-SeT, Coris BioConcept, Gembloux, Belgium) on a short-term culture. Additionally, we validated the OXA-48 K-SeT and Xpert Carba-R assay (Cepheid, Sunnyvale, CA) on subcultures of positive blood cultures that were incubated shortly (6 h).

Case

We present a case of a 56-year-old Caucasian male who underwent a second kidney transplant in November 2023. Shortly thereafter, the patient developed acute pyelonephritis caused by *Klebsiella oxytoca*, treated initially with temocillin IV (2 g twice per day) and subsequently with amoxicillin-clavulanic acid orally (875 mg/125 mg three times per day) for a total of 14 days.

In January 2024, the patient presented to the emergency department with fever, which was attributed to recurrent pyelonephritis. Levofloxacin (500 mg once daily), started 3 weeks earlier in the context of an orthopedic infection, was continued. Two sets of blood cultures and a urine culture

✉ Jerina Boelens
jerina.boelens@uzgent.be

1 Department of Medical Microbiology, Ghent University Hospital, Ghent, Belgium

2 Department of Internal Medicine and Infectious Diseases, Ghent University Hospital, Ghent, Belgium

3 Department of Intensive Care Medicine, Ghent University Hospital, Ghent, Belgium

4 Department of Internal Medicine and Paediatrics, Ghent University Hospital, Ghent, Belgium

5 Department of Diagnostic Sciences, Ghent University, Ghent, Belgium

Published online: 19 December 2024

Table 1 Antimicrobial susceptibility results

Antibiotic	MIC Breakpoints		ASTar results		Zone diameter breakpoints		Disk diffusion results	
	S ≤	*R* >	MIC	SIR	S ≤	*R* >	Ø	SIR
Amoxicillin-clavulanic acid	8	8	> 32	R	19	19	6	R
Piperacillin/tazobactam	8	8	256	R	20	20	9	R
Temocillin	0,001	16	-	-	50	17	6	R
Ceftriaxone	1	2	> 128	R	20	17	6	R
Ceftazidime	1	4	> 64	R	22	19	6	R
Ceftazidime/avibactam	8	8	0,25	S	13	13		-
Meropenem	2	8	2	S	22	16	21	I
Ertapenem	0.5	0.5	2	R	25	25	-	-
Ofloxacin	0,25	0,5	-	-	24	22	6	R
Levofloxacin	0.5	1	> 16	R	23	19	-	-
Amikacin	8	8	4	S	18	18	19	S
Aztreonam	1	4	64	R	26	21	11	R
Gentamicin	2	2	> 32	R	17	17	-	-
Tobramycin	2	2	> 32	R	16	16	-	-
Trimethoprim/sulfamethoxazole	2	4	> 8	R	14	11	6	R

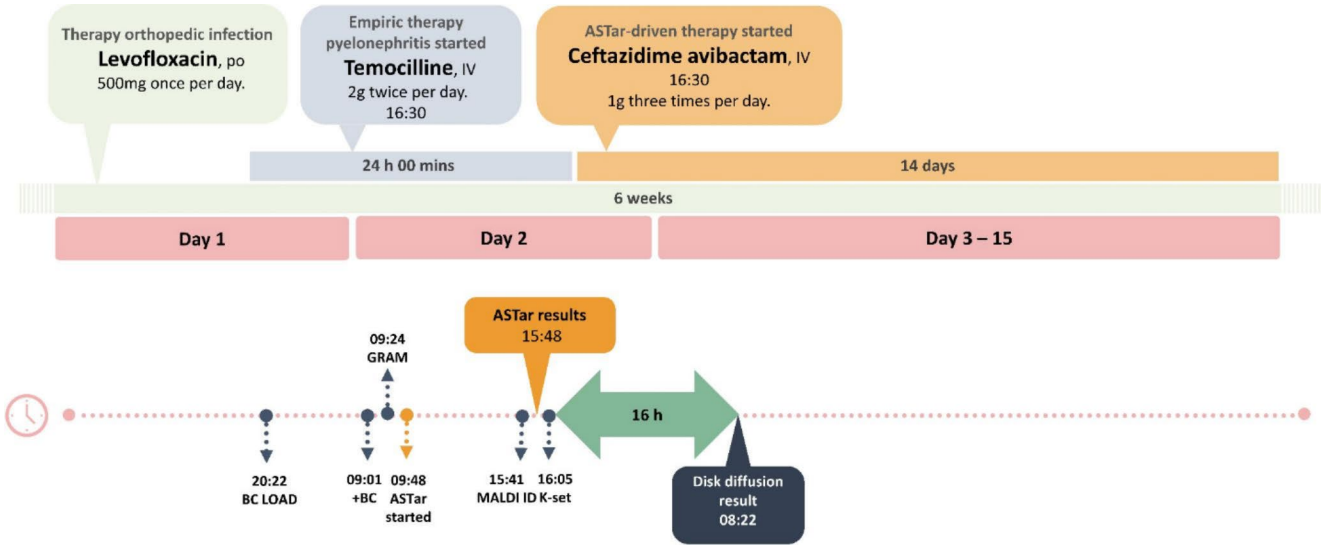

Fig. 1 Timeline of antibiotic therapy and laboratory results

were taken at the emergency department, and the patient was admitted to the general ward. Therapy with IV temocillin was added for suspicion of pyelonephritis and a second set of blood cultures was collected at the time of fever.

The blood cultures were incubated in the automated BACTEC FX system (Becton Dickinson GmbH, Heidelberg, Germany). The two aerobic bottles of the last set flagged positive after 12 h. Gram-stain showed Gram-negative rods. Subcultures were made on a blood and MacConkey agar (BD) for overnight incubation and a short-term subculture was incubated on a blood agar for rapid identification after 5 h according to the method described by Verroken et al. [2]. Direct AST was done with the disk diffusion method according to EUCAST guidelines [3]. Additionally rapid AST was performed using the ASTar system, which performs fully automated microdilution AST directly from positive blood cultures with Gram-negative micro-organisms, in about 6 h [4].

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) of the short-term subculture identified the Gram-negative rods in the blood culture as *Klebsiella pneumoniae*. The ASTar-system revealed a MIC of meropenem and ertapenem of 2 mg/L (Table 1), which is above the screening cut-off for CPE according to EUCAST [1]. The system does not test temocillin susceptibility, however. An OXA-48 *K*-SeT to detect OXA-48 was performed on the short-term subculture and was positive. Since OXA-48 type CPE is associated with high level resistance to temocillin [5], the therapy of the patient was urgently switched to ceftazidime-avibactam (1 g/0.25 g three times per day) and the patient was isolated. Figure 1 shows the timeline of antibiotic therapy and

Table 2 Validation of OXA-48 *K*-SeT and Xpert Carba-R assay on short-term culture

Isolate	Bactec Plus Aerobic/F	Bactec anaerobic/F lytic	Bactec Peds Plus/F
OXA-48 K-SeT			
KPC *K. pneumoniae*	Negative at 2, 4, 6 h	Negative at 2, 4, 6 h	NP
OXA-48 K. pneumoniae	Positive at 2, 4, 6 h	Positive at 2, 4, 6 h	NP
OXA-48 K. pneumoniae	Positive at 2, 4, 6 h	Positive at 2, 4, 6 h	Positive at 2, 4, 6 h
OXA-48 K. pneumoniae	NP	Positive at 2, 4, 6 h	Positive at 2, 4, 6 h
GeneXpert			
KPC *K. pneumoniae*	NP	Positive: KPC	NP
IMP K. pneumoniae	Positive: IMP	Positive: IMP	NP
VIM K. pneumoniae	Positive: VIM	NP	NP
NDM + OXA-48 K. pneumoniae	Positive: NDM + OXA-48	NP	NP
ESBL K. pneumoniae	Negative	NP	NP

We spiked blood cultures with multi-drug-resistant Gram-negative isolates from reference isolates and isolates from clinical routine according to Cumitech 31 A and 5 mL of human left-over blood leading to a bacterial suspension of ± 40 CFU/mL of blood [6]. We used aerobic (BD BACTEC Plus Aerobic/F), anaerobic (BD BACTEC Plus Anaerobic/F) and pediatric bottles (BACTEC Peds Plus/F). The bottles were incubated in BACTEC FX system. When the bottles flagged positive, a subculture was made on a blood agar which was incubated at 37 °C in a 5% supplemented CO_2 atmosphere. OXA-48 *K*-SeT was performed on the subculture at 2 h, 4 h and 6 h. Xpert Carba-R assay was only tested at 6 h. The table shows which isolates were incubated in which bottle and the results of the tests. No tests were performed (NP) when the isolate was not incubated in the corresponding bottle

laboratory results. The next day, resistance to temocillin was confirmed by the disc diffusion method. We additionally performed the Xpert Carba-R assay on mature colonies, which was positive for OXA-48. Urine culture was also positive for OXA-48 type *K. pneumoniae*. The patient was treated 14 days with ceftazidime-avibactam with good clinical response.

Following this case, we performed a validation study to determine whether confirmatory CPE tests such as LFA or molecular assay can be reliably performed on short-term subcultures from positive blood cultures. Table 2 shows the validation protocol as well as the results of the validation study of OXA-48 *K*-SeT and Xpert Carba-R assay on short term cultures.

Conclusion

We present a case where OXA-48 producing *K. pneumoniae* with low-level resistance to carbapenems was suspected early using RAST with ASTar. This allowed for an early switch to effective antimicrobial therapy in this immunocompromised patient. RAST for blood cultures may require confirmation for CPE with rapid detection tests. Our validation study showed that the OXA-48 K-SeT was consistently positive for OXA-48 isolates at 2, 4, and 6 h and negative for other CPE, while the Xpert Carba-R assay accurately identified OXA-48, NDM, VIM, KPC, and IMP isolates on subcultures from positive blood cultures after 6 h. These findings support the use of OXA-48 K-SeT and Xpert Carba-R assays on short-term cultures for rapid and accurate detection of CPE in clinical practice.

Author contributions Material preparation, data collection and analysis were performed by E. De Muynck and J. Boelens. The first draft of the manuscript was written by E. De Muynck and all authors commented on previous versions of the manuscript. Emilie De Muynck, Stien Vandendriessche, Anne-Sophie Messiaen, Kristen Decommer, Diana Huis in't Veld, Jan J. De Waele and Jerina Boelens all read and approved the final manuscript.

Funding The authors declare that no funds or grants were received during the preparation of this manuscript. Q-linea provided the ASTar-system and kit used, but the company had no role in data collection and analysis, the decision to publish, or the preparation of the manuscript.

Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

References

1. The European Committee on Antimicrobial Susceptibility Testing (2013) EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance, version 1.0

2. Verroken A, Defourny L, Lechgar L, Magnette A, Delmée M, Glupczynski Y (2015) Reducing time to identification of positive blood cultures with MALDI-TOF MS analysis after a 5-h subculture. Eur J Clin Microbiol Infect Dis 34(2):405–413. https://doi.org/10.1007/s10096-014-2242-4
3. The European Committee on Antimicrobial Susceptibility Testing (2024) Antimicrobial susceptibility testing EUCAST disk diffusion method, version 12.0
4. Esse J, Träger J, Valenza G, Bogdan C, Held J (2023) Rapid phenotypic antimicrobial susceptibility testing of Gram-negative rods directly from positive blood cultures using the novel Q-linea ASTar system. J Clin Microbiol 61(11):e0054923. https://doi.org/10.1128/jcm.00549-23
5. 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 69(2):445–450. https://doi.org/10.1093/jac/dkt367
6. Clark RB, Lewinski MA, Loeffelholz MJ, Tibbets RJ (2009) Cumitech 31A Verification and validation of Procedures in the Clinical Microbiology Laboratory