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The congress of ESCMID

Rapid Detection of *Escherichia coli* and *Klebsiella pneumoniae* and Resistance Markers in Positive Blood Cultures by Using MALDI BioTyper System and FilmArray Blood Culture Identification Panel combined with the Eazyplex SuperBug CRE assay

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Extended spectrum β-lactamase producing

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MINIREVIEW

Carbapenemase-Producing *Klebsiella pneumoniae*, a Key Pathogen Set for Global Nosocomial Dominance

Johann D. D. Pitout,[a,b,c,d] Patrice Nordmann,[e,f] Laurent Poirel[f]

TABLE 1 Characteristics of *K. pneumoniae* strains that produce carbapenemases

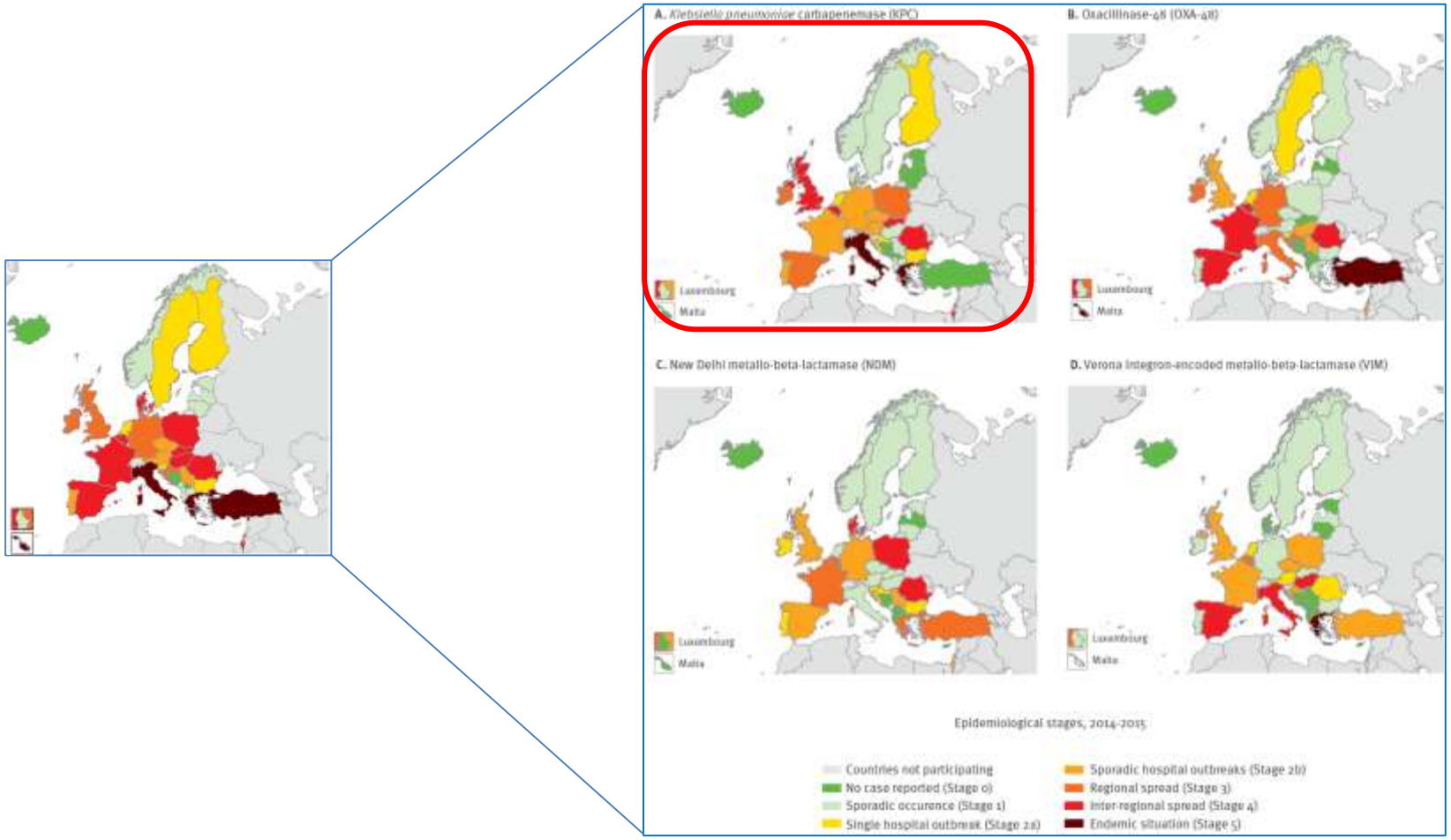
Enzyme types (class) and examples	Spectrum of activity	Inhibitor(s)	Areas of endemicity	Molecular epidemiology
MBLs (B): NDM-1, IMP, VIM	Penicillins, cephalosporins, cephamycins, carbapenems	Metal chelators, e.g., EDTA, dipicolinic acid	Japan (IMP), Taiwan (IMP), Indian subcontinent (NDM), Balkan states (NDM), Greece (VIM)	IncA/C, N plasmids (NDM), class I integrons (VIM, IMP)
KPCs (A): KPC-2, -3, others	Penicillins, cephalosporins, cephamycins, carbapenems	Clavulanic acid (weak), tazobactam(weak), boronic acid, avibactam	United States, Greece, Italy, Israel, China, Brazil, Colombia, Argentina	Tn*4401*, IncFII plasmids, CC258
OXA-β-lactamases (D): OXA-48, OXA-181, OXA-204, OXA-232	Penicillins, temocillin, β-lactamase inhibitor combinations, carbapenems (weak)	NaCl	Turkey, North Africa (Morocco, Tunisia), Europe (Spain, Belgium)	Tn*1999*, IncL/M plasmids

Klebsiella pneumoniae: the CTX-M-15 type consolidation

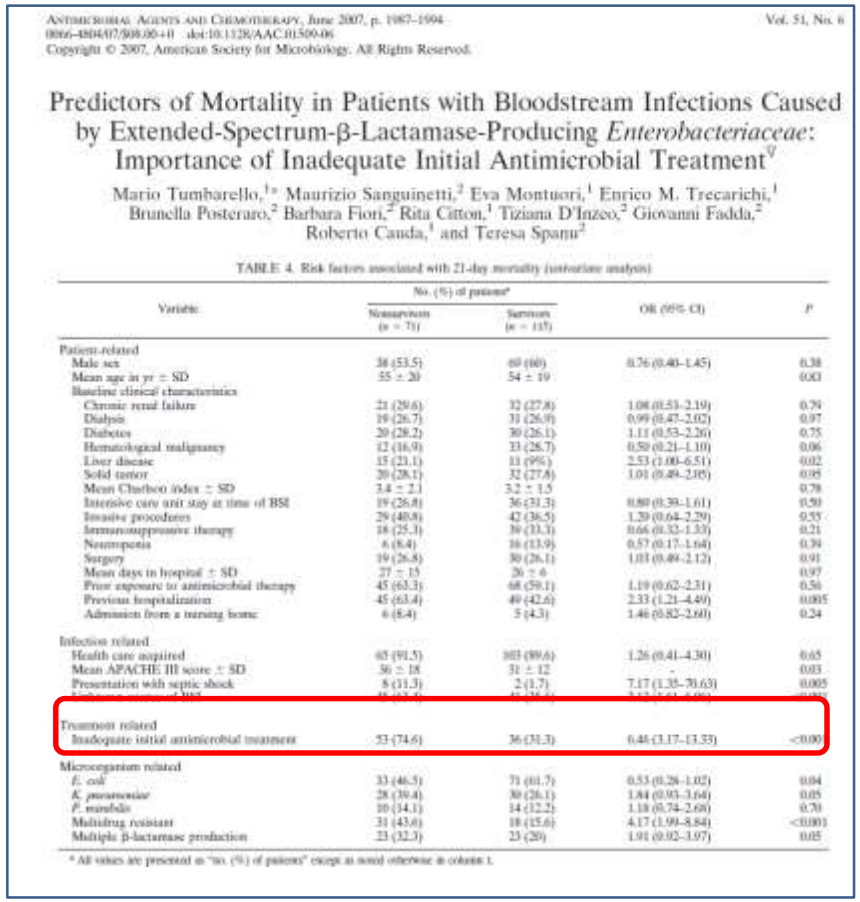
Esther Calbo[1,2] & Javier Garau[3,4] *Future Microbiol.* (2015) 10(6), 1063–1075

Carbapenemase-producing *Enterobacteriaceae* in Europe: assessment by national experts from 38 countries, May 2015

B Albiger [1], C Glasner [2 3], MJ Struelens [1], H Grundmann [2], DL Monnet [1], the European Survey of Carbapenemase-Producing Enterobacteriaceae (EuSCAPE) working group [4]



Geographic distribution of carbapenemase-producing *Enterobacteriaceae* by resistance mechanism, based on self-assessment by national experts, 38 European countries, May 2015



ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 2007, p. 1987–1994 Vol. 51, No. 6
0066-4804/07/$08.00+0 doi:10.1128/AAC.01509-06

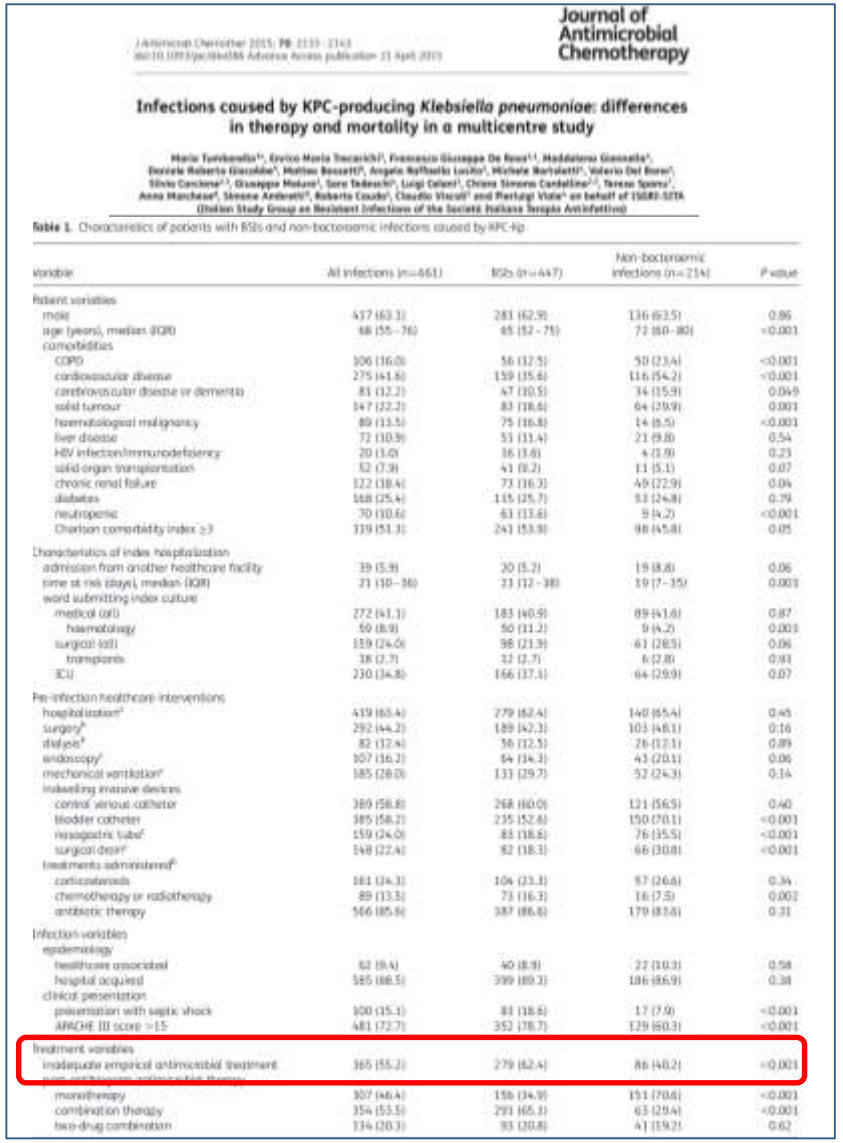
Predictors of Mortality in Patients with Bloodstream Infections Caused by Extended-Spectrum-β-Lactamase-Producing *Enterobacteriaceae*: Importance of Inadequate Initial Antimicrobial Treatment▽

Mario Tumbarello,[1]* Maurizio Sanguinetti,[2] Eva Montuori,[1] Enrico M. Trecarichi,[1] Brunella Posteraro,[2] Barbara Fiori,[2] Rita Citton,[1] Tiziana D'Inzeo,[2] Giovanni Fadda,[2] Roberto Cauda,[1] and Teresa Spanu[2]

TABLE 4. Risk factors associated with 21-day mortality (univariate analysis)

Variable	No. (%) of patients* Nonsurvivors (n = 71)	Survivors (n = 115)	OR (95% CI)	P
Patient-related				
Male sex	38 (53.5)	69 (60)	0.76 (0.40–1.45)	0.38
Mean age in yr ± SD	55 ± 20	54 ± 19		0.03
Baseline clinical characteristics				
Chronic renal failure	21 (29.6)	32 (27.8)	1.08 (0.53–2.19)	0.79
Dialysis	19 (26.7)	31 (26.9)	0.99 (0.47–2.02)	0.97
Diabetes	20 (28.2)	30 (26.1)	1.11 (0.53–2.26)	0.75
Hematological malignancy	12 (16.9)	33 (28.7)	0.50 (0.21–1.10)	0.06
Liver disease	15 (21.1)	11 (9%)	2.53 (1.00–6.51)	0.02
Solid tumor	20 (28.1)	32 (27.8)	1.01 (0.49–2.05)	0.95
Mean Charlson index ± SD	3.4 ± 2.1	3.2 ± 1.5		0.78
Intensive care unit stay at time of BSI	19 (26.8)	36 (31.3)	0.80 (0.39–1.61)	0.50
Invasive procedures	26 (40.8)	42 (36.5)	1.20 (0.64–2.29)	0.55
Immunosuppressive therapy	18 (25.3)	39 (33.3)	0.66 (0.32–1.33)	0.21
Neutropenia	6 (8.4)	16 (13.9)	0.57 (0.17–1.64)	0.34
Surgery	19 (26.8)	30 (26.1)	1.03 (0.49–2.12)	0.91
Mean days in hospital ± SD	27 ± 15	26 ± 6		0.97
Prior exposure to antimicrobial therapy	45 (63.3)	68 (59.1)	1.19 (0.62–2.31)	0.56
Previous hospitalization	45 (63.4)	49 (42.6)	2.33 (1.21–4.49)	0.005
Admission from a nursing home	6 (8.4)	5 (4.3)	1.46 (0.82–2.60)	0.24
Infection related				
Health care acquired	65 (91.5)	103 (89.6)	1.26 (0.41–4.30)	0.65
Mean APACHE III score ± SD	56 ± 18	51 ± 12		0.03
Presentation with septic shock	8 (11.3)	2 (1.7)	7.17 (1.35–70.63)	0.005
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Treatment related				
Inadequate initial antimicrobial treatment	53 (74.6)	36 (31.3)	0.46 (3.17–13.33)	<0.001
Microorganism related				
E. coli	33 (46.5)	71 (61.7)	0.53 (0.28–1.02)	0.04
K. pneumoniae	28 (39.4)	30 (26.1)	1.84 (0.93–3.64)	0.05
P. mirabilis	10 (14.1)	14 (12.2)	1.18 (0.74–2.68)	0.70
Multidrug resistant	31 (43.6)	18 (15.6)	4.17 (1.99–8.84)	<0.001
Multiple β-lactamase production	23 (32.3)	23 (20)	1.91 (0.92–3.97)	0.05

* All values are presented as "no. (%) of patients" except as noted otherwise in column 1.



Journal of Antimicrobial Chemotherapy

Infections caused by KPC-producing *Klebsiella pneumoniae*: differences in therapy and mortality in a multicentre study

Mario Tumbarello[1]*, Enrico Maria Trecarichi[1], Francesco Giuseppe De Rosa[1,2], Maddalena Giannella[3], Daniele Roberto Giacobbe[4], Matteo Bassetti[4], Angelo Raffaele Lucio[5], Michele Bartoletti[3], Valerio Del Bono[6], Silvia Corcione[1,2], Giuseppe Maiuro[1], Sara Tedeschi[3], Luigi Celani[1], Chiara Simona Cardellino[1,2], Teresa Spanu[7], Anna Marchese[8], Simone Anderini[9], Roberto Cauda[1], Claudio Viscoli[4] and Pierluigi Viale[3] on behalf of ISGRI-SITA (Italian Study Group on Resistant Infections of the Società Italiana Terapia Antinfettivi)

Table 1. Characteristics of patients with BSIs and non-bacteraemic infections caused by KPC-Kp

Variable	All infections (n = 661)	BSIs (n = 447)	non-bacteraemic infections (n = 214)	P value
Patient variables				
male	417 (63.1)	281 (62.9)	136 (63.5)	0.86
age (years), median (IQR)	68 (55–76)	65 (52–75)	72 (60–80)	<0.001
comorbidities				
COPD	106 (16.0)	56 (12.5)	50 (23.4)	<0.001
cardiovascular disease	275 (41.6)	159 (35.6)	116 (54.2)	<0.001
cerebrovascular disease or dementia	81 (12.2)	47 (10.5)	34 (15.9)	0.049
solid tumour	147 (22.2)	83 (18.6)	64 (29.9)	0.001
haematological malignancy	89 (13.5)	75 (16.8)	14 (6.5)	<0.001
liver disease	72 (10.9)	51 (11.4)	21 (9.8)	0.54
HIV infection/immunodeficiency	20 (3.0)	16 (3.6)	4 (1.9)	0.23
solid organ transplantation	52 (7.9)	41 (9.2)	11 (5.1)	0.07
chronic renal failure	122 (18.4)	73 (16.3)	49 (22.9)	0.04
diabetes	168 (25.4)	115 (25.7)	53 (24.8)	0.79
neutropenic	70 (10.6)	61 (13.6)	9 (4.2)	<0.001
Charlson comorbidity index ≥3	339 (51.3)	241 (53.9)	98 (45.8)	0.05
Characteristics of index hospitalization				
admission from another healthcare facility	39 (5.9)	20 (5.2)	19 (8.8)	0.06
time at risk (days), median (IQR)	21 (10–36)	21 (12–38)	19 (7–35)	0.003
ward submitting index culture				
medical (all)	272 (41.1)	183 (40.9)	89 (41.6)	0.87
haematology	59 (8.9)	50 (11.2)	9 (4.2)	0.003
surgical (all)	159 (24.0)	98 (21.9)	61 (28.5)	0.06
transplants	18 (2.7)	12 (2.7)	6 (2.8)	0.93
ICU	230 (34.8)	166 (37.1)	64 (29.9)	0.07
Pre-infection healthcare interventions				
hospitalization[a]	419 (63.4)	279 (62.4)	140 (65.4)	0.45
surgery[b]	292 (44.2)	189 (42.3)	103 (48.1)	0.16
dialysis[b]	82 (12.4)	56 (12.5)	26 (12.1)	0.89
endoscopy[c]	107 (16.2)	64 (14.3)	43 (20.1)	0.06
mechanical ventilation[c]	185 (28.0)	133 (29.7)	52 (24.3)	0.14
indwelling invasive devices				
central venous catheter	389 (58.8)	268 (60.0)	121 (56.5)	0.40
bladder catheter	385 (58.2)	235 (52.6)	150 (70.1)	<0.001
nasogastric tube[c]	159 (24.0)	83 (18.6)	76 (35.5)	<0.001
surgical drain[c]	148 (22.4)	82 (18.3)	66 (30.8)	<0.001
treatments administered[b]				
corticosteroids	161 (24.3)	104 (23.3)	57 (26.6)	0.34
chemotherapy or radiotherapy	89 (13.5)	73 (16.3)	16 (7.5)	0.002
antibiotic therapy	566 (85.6)	387 (86.6)	179 (83.6)	0.31
Infection variables				
epidemiology				
healthcare associated	62 (9.4)	40 (8.9)	22 (10.3)	0.58
hospital acquired	585 (88.5)	399 (89.3)	186 (86.9)	0.38
clinical presentation				
presentation with septic shock	100 (15.1)	83 (18.6)	17 (7.9)	<0.001
APACHE III score >15	481 (72.7)	352 (78.7)	129 (60.3)	<0.001
Treatment variables				
inadequate empirical antimicrobial treatment	365 (55.2)	279 (62.4)	86 (40.2)	<0.001
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
monotherapy	307 (46.4)	156 (34.9)	151 (70.6)	<0.001
combination therapy	354 (53.5)	291 (65.1)	63 (29.4)	<0.001
two-drug combination	134 (20.3)	93 (20.8)	41 (19.2)	0.62

American Journal of Hematology AJH

Research Article

Bloodstream infections caused by *Klebsiella pneumoniae* in onco-hematological patients: clinical impact of carbapenem resistance in a multicentre prospective survey

Am J Hematol. 2016 Nov;91(11):1076-1081. doi: 10.1002/ajh.24489. Epub 2016 Jul 29.

Rapid and accurate detection of these pathogens is a prerequisite for successful infection management, for monitoring resistance trends and implementation of intervention strategies.

Lab algorithm for detection and identification of bloodstream pathogens

0h

Detection of microbial growth

Gram stain

1h

Direct MALDI-TOF MS

Culture-based method

If polimicrobial or no ID

Call to infectious disease consultants

Film Array BC panel

Antimicrobial susceptibility testing

If *K.pneumoniae* or *E.coli*

24h

Eazyplex SuperBug CRE System

Lab results

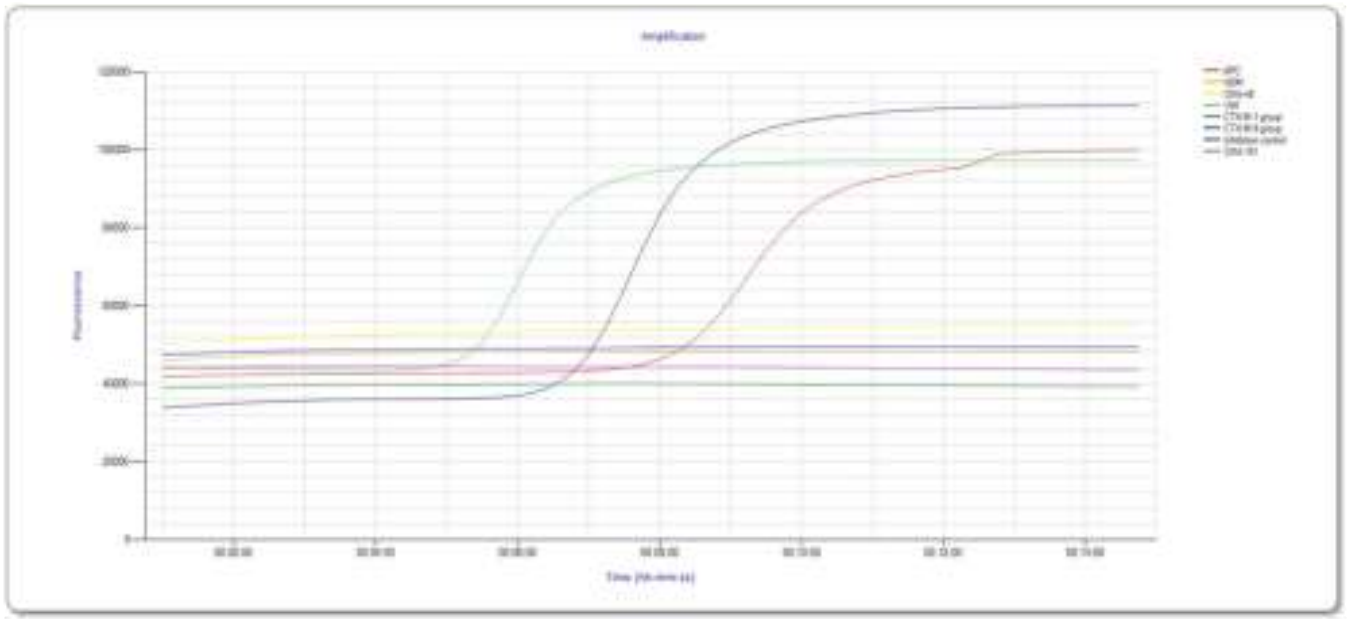
24h

Lab results

The **eazyplex® SuperBug CRE** is a qualitative genotypic diagnostic test able to provide a result in 20 min.

The clinical experience, to date, shows that this panel identifies **CTX-M-1** and **CTX-M-9** ESBL families and carbapenemase variants of the **VIM** (-1 to -37), **NDM** (1- to -7), **KPC** (-1 to -15) families, **OXA-48-like** (-48, -162, -204, and-244) and **OXA-181** with high accuracy when applied directly from single bacterial colonies grown on agar plates and urine samples.

	Parameter	Result
A1	KPC	positive
A2	NDM	negative
A3	OXA-48	negative
A4	VIM	positive
A5	CTX-M 1 group	negative
A6	CTX-M 9 group	negative
A7	Inhibition control	valid
A8	OXA-181	negative



Amplification plot of *bla*$_{KPC}$ and *bla* $_{VIM}$ positive in a blood culture sample containing *Klebsiella pneumoniae* isolate

Evaluation of three commercial assays for rapid detection of genes encoding clinically relevant carbapenemases in cultured bacteria. García- Findlay J et al. J Antimicrob Chemother 70(5):1338-42. 2015

Evaluation of the eazyplex® SuperBug CRE system for rapid detection of carbapenemases and ESBLs in clinical Enterobacteriaceae isolates recovered at two Spanish hospitals. Fernández S et al. J Antimicrob Chemother 70:1047-50. 2015.

Extended-spectrum β-lactamase (ESBL) detection directly from urine samples with the rapid isothermal amplification-based eazyplex® SuperBug CRE assay: proof of concept. Hinić V et al. J Microbiol Methods 119:203-5. 2015.

Aim of the study:

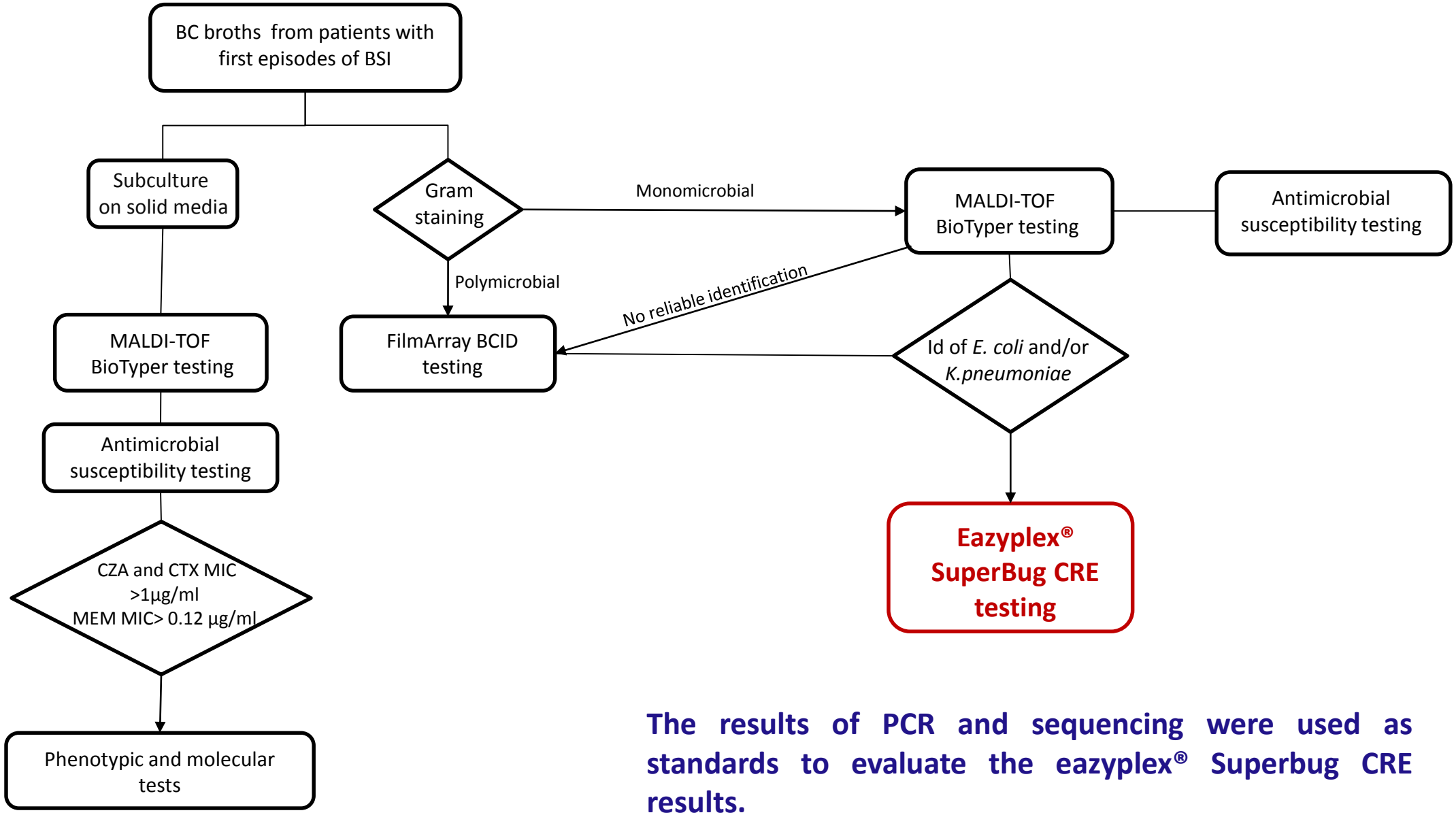
In the present 1-year analysis of consecutive EC- and/or KP-BSIs, we explored the diagnostic performance of our rapid BC strategy

- **Retrospective study : BCs spiked with 126 well characterized strains including 34 harbouring carbapenemase genes, 40 harbouring ESBL genes and negative control strains.**

- **Prospective study: from January 2015 to January 2016 clinical BCs Implementation in the routine lab.**

BCs, blood cultures; BSIs, bloodstream infections; EC, *E. coli*, KP, *K. pneumoniae*

Methods:



The results of PCR and sequencing were used as standards to evaluate the eazyplex® Superbug CRE results.

CZA, Ceftazidime; CTX, Cefotaziime; MEM, Meropenem

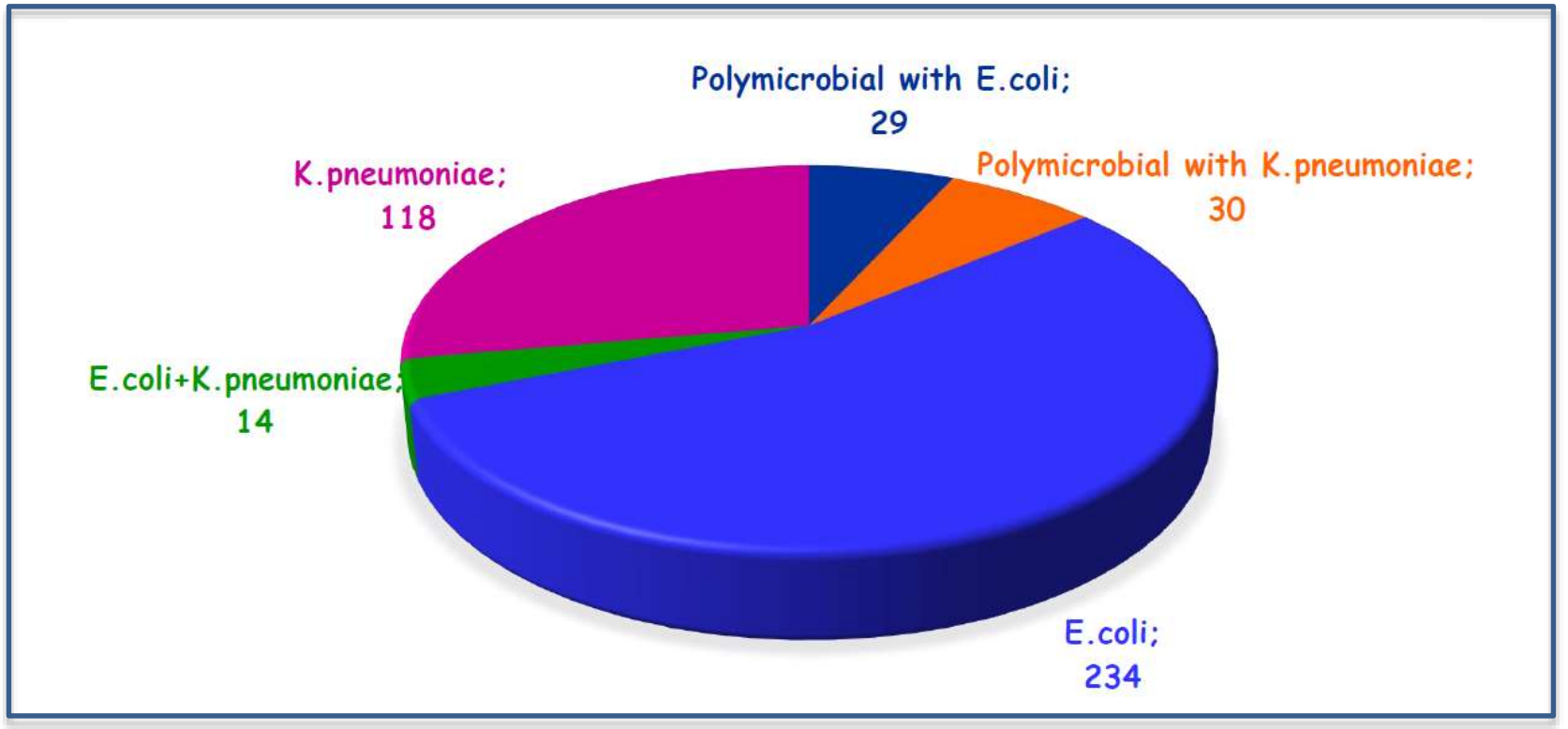
Eazyplex® SuperBug CRE Results for126 spiked blood cultures :

	C. freundii (1)	*E. cloacae* (3)	*E. coli* (79)	*K. oxytoca* (1)	*K.pneumoniae* (42)
CTX-M1	-	-	23	-	5
CTX-M9	-	-	12	-	-
KPC	-	-	4	-	15
KPC+ CTX-M-1	-	-	-	-	2
KPC+VIM	-	-	-	-	3
NDM	-	-	1	-	1
OXA-48	1	1	1	-	-
VIM	-	2	1	-	1
NDM+ CTX-M-1	-	-	1	-	-
NEG	-	-	36	1	15

100% of Concordance with the comparison method.

Culture based results for 425 BCs:

425 positive blood cultures: **352 monomicrobial**
73 polymicrobial (116 Gram neg. 36 Gram pos. and 6 Yeasts**)**

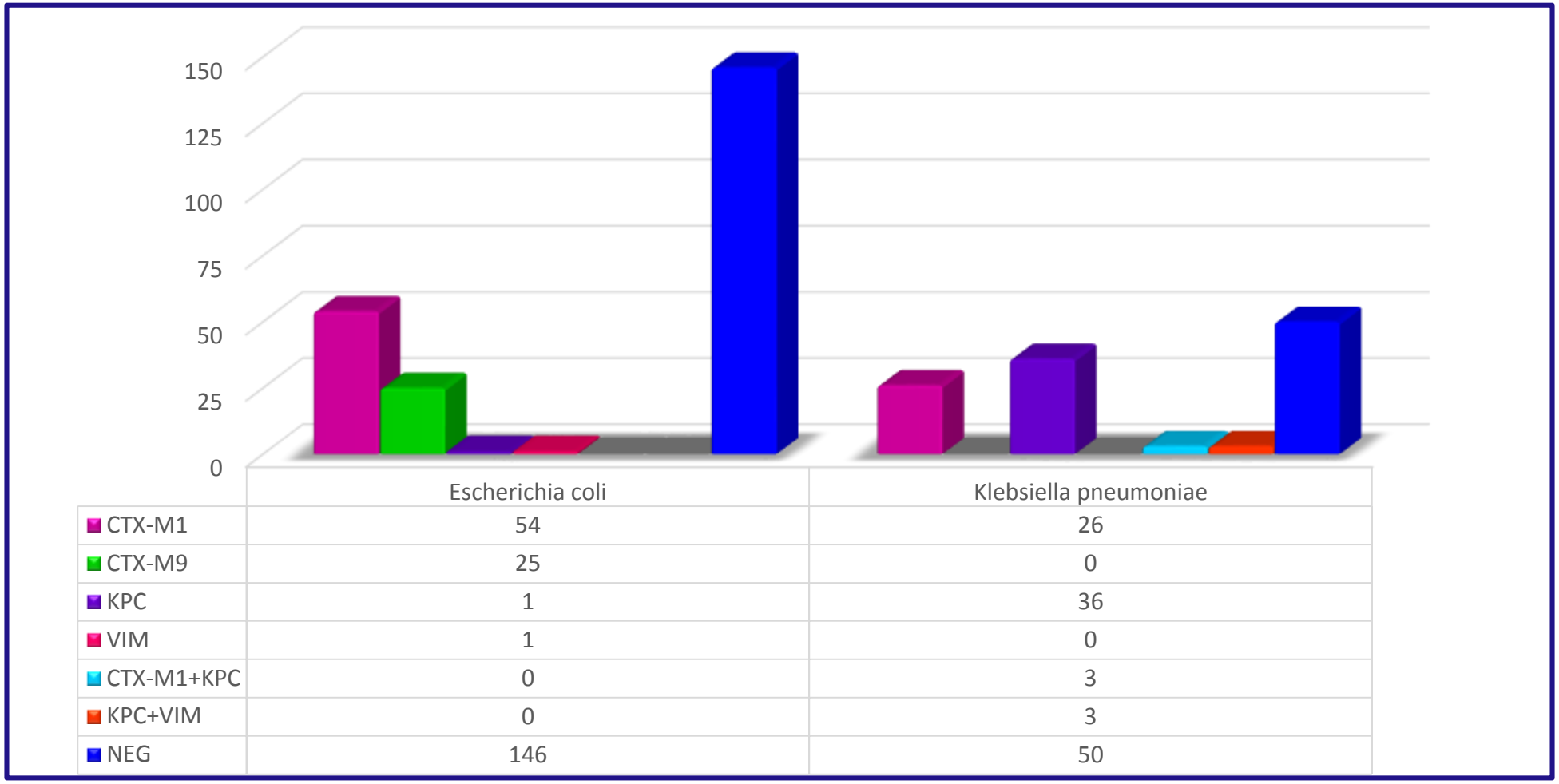


The median TTD for the 425 growth-positive BCs was 8.3 hours (IQR: 4.6-11.5).

- **ESBL production was detected in 28.5% (125/439) of the isolates [33.6% (93/277) of EC and 19.7% (32/162) of KP],** and all carried a CTX-M ESBL gene. Isolates carrying $bla_{\text{CTXM-15}}$ were the most prevalent (*n*=94, 75.2%) followed by strains producing CTX-M-27 (*n*=15, 12%), CTX-M-14 (*n*=11, 8.8%), and CTX-M-1 (*n*=5, 4.0%). The $bla_{\text{CMY-2}}$ gene was detected in 1.6% of the isolates (all EC).
- **Carbapenemase-encoding genes were found in 34.6% (56/162) of the KP isolates and in 1.1% (3/277) of EC** isolates being $bla_{\text{KPC-3}}$ the most common detected gene.

BCs, blood cultures; TTD, time to detection BSIs, bloodstream infections; EC, *E. coli*, KP, *K. pneumoniae*

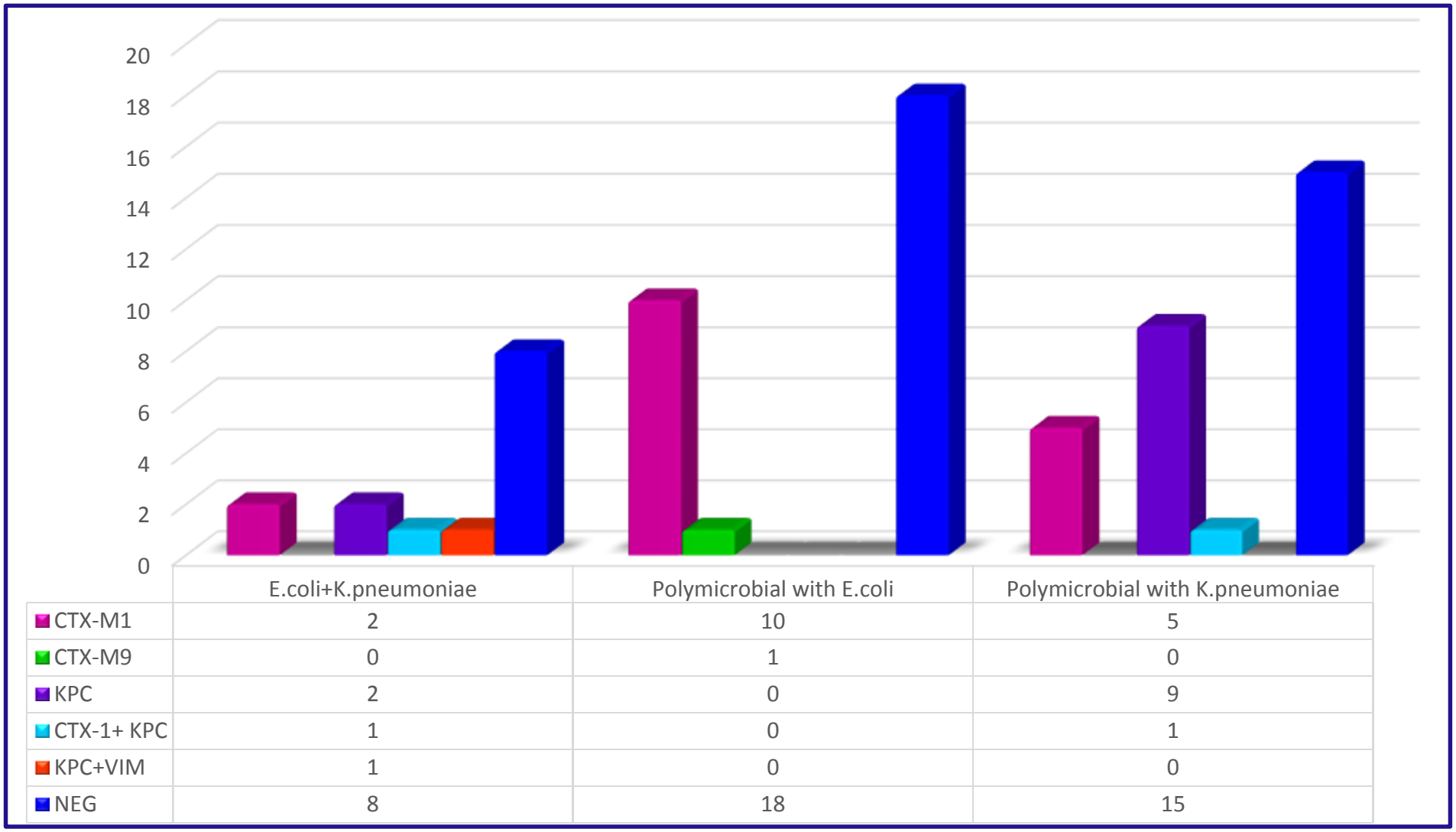
Eazyplex® SuperBug CRE Results for 352 monomicrobial BCs:



	Escherichia coli	Klebsiella pneumoniae
CTX-M1	54	26
CTX-M9	25	0
KPC	1	36
VIM	1	0
CTX-M1+KPC	0	3
KPC+VIM	0	3
NEG	146	50

All the KPC, VIM and CTX-M genes were correctly detected, with no false-positives, yielding sensitivity and specificity for each target of 100% and 100%, respectively. The median TTR was 18 hours by using the direct method compared to 46 hours by using the conventional method ($P<0.001$).

Eazyplex® SuperBug CRE Results for 73 polymicrobial BCs:



	E.coli+K.pneumoniae	Polymicrobial with E.coli	Polymicrobial with K.pneumoniae
CTX-M1	2	10	5
CTX-M9	0	1	0
KPC	2	0	9
CTX-1+ KPC	1	0	1
KPC+VIM	1	0	0
NEG	8	18	15

The Eazyplex displayed 100 % of concordance with the comparison method for detection of CTX-M and carbapenemase genes.

Additionally, the FilmArray BCID panel correctly reported 14 positive *bla*$_{KPC}$ results among the 73 polymicrobial BCs evaluated.

Conclusions

- Further studies are needed to evaluate its overall performance, our experience with this large series of EC and KP BSIs indicates that the flowchart we evaluated is a reliable time-saving tool for routine ID of isolates producing CTX-M ESBLs and KPC and VIM carbapenemases.

- It furnished results in a median time of 18 h after BC entry in the automated systems, and coupled with Antimicrobial Stewardship intervention could improved antibiotic utilization in early course of the illness.

- Further study is needed to confirm this model's potential as a guide for prescribing early effective antibiotic therapy of patients with CTX-M-BSI and KPC-BSI.

- The major drawback of our procedure is the inability to detect ESBLs belong to the TEM and SHV families, pAmpC genes, and some OXA-48–like variants, which are found among currently spreading MDR members of the *Enterobacteriaceae.* Therefore without the ability to detect such determinants, the eazyplex® Superbug CRE assay would miss a significant proportion of strains if implemented in settings where microorganisms carrying those genes are frequent. Future improvements may enhance the assay’s benefit.