International Journal of Antimicrobial Agents 53 (2019) 268–274

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: www.elsevier.com/locate/ijantimicag

Detection and prevalence of carbapenem-resistant Gram-negative bacteria among European laboratories in the COMBACTE network: a COMBACTE LAB-Net survey

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ARTICLE INFO

Article history:
Received 7 June 2018
Accepted 23 October 2018

Editor: A Tsakris

Key words:
Carbapenem resistance
Questionnaire
Clinical trials

ABSTRACT

Antimicrobial resistance (AMR) represents a global public health threat that jeopardises the progress medicine has made over the last century. To confront AMR, the Innovative Medicines Initiative (IMI) has supported the development of a large network of hospitals and laboratories in Europe as part of the New Drugs for Bad Bugs (ND4BB) programme and the COMBACTE projects. COMBACTE LAB-Net conducted a pilot survey on distribution and usage of carbapenem resistance detection methods among laboratories in the COMBACTE network in two clinical trials as part of the COMBACTE-CARE project. The survey was sent out to 211 laboratories in 20 European countries between May 2015 and June 2017. Answers were collected from 165 laboratories (78%). Sixty laboratories (36%) reported an outbreak of carbapenem-resistant (CR) Enterobacteriaceae during one of the two years preceding the completion of the survey. High rates of CR *Acinetobacter* spp. above 50% were reported by 74 laboratories (47%), particularly in the Western Balkan countries where the rates were sometimes higher than 90%. Apart from determining the antimicrobial susceptibility of isolates, laboratories also used various methods, such as Matrix Assisted Laser Desorption Ionization - Time of Flight (MALDI-TOF), Carbapenemase Nordmann-Poirel (Carba NP) test or molecular methods, to detect CR Gram-negative bacteria. The survey resulted in the selection of sites with high resistance rates that successfully recruited many patients in the EURECA observational clinical trial.

1. Introduction

Antimicrobial resistance (AMR) causes numerous deaths globally, diminishes quality of life, and threatens to reverse much of the progress modern medicine has made over the last century. Unless action is taken, up to an estimated ten million additional lives will be lost prematurely each year because of AMR by 2050 [1,2]. Prudent use of existing antibiotics and accelerated development of new antimicrobial agents are desperately needed to preserve the effectiveness of these "wonder drugs". Some bacterial species have developed resistance against all useful human antibiotics used extensively in the management of infections caused by multidrug-resistant (MDR) pathogens. However, it is already abundantly clear that focussing on discovering new antibiotics alone will not avert this current, growing crisis any time soon. Accurate and rapid laboratory diagnostics are equally important to ensure timely detection of pathogens and optimisation of anti-infective therapy [3,4]. In both routine diagnostics and anti-infective clinical trials, it is crucial for the microbiology laboratory to rapidly provide evidence of the presence of MDR pathogens in clinical samples. A particular concern is the emergence of carbapenemase-producing colistin-resistant Gram-negative bacteria, which have developed resistance against all useful human antibiotics. Several phenotypic tests are widely used to detect carbapenem-resistant Gram-negative bacteria (CR-GNB), including chromogenic media, Modified Hodge test, Carbapenemase Nordmann-Poirel (Carba NP) test, Blue-carba test, carbapenem inactivation method, and mass spectrometry-based Matrix Assisted Laser Desorption Ionization - Time of Flight (MALDI-TOF) [5-8]. Unfortunately, some of these non-molecular tests lack sensitivity or specificity, are time-consuming [8], or fail to detect certain types of CR-GNB, e.g., OXA-48-producing Enterobacteriaceae [9]. They are often combined with or replaced

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https://doi.org/10.1016/j.ijantimicag.2018.10.013

by rapid molecular diagnostic tests, such as GeneXpert Carba-R and in-house multiplex polymerase chain reaction (PCR).

The Innovative Medicines Initiative (IMI) has supported the development of a large network of hospitals and laboratories in Europe as part of the New Drugs for Bad Bugs (ND4BB) programme and the Combatting bacterial resistance in Europe (COMBACTE) projects [10]. By December 2017, more than 650 laboratories in 41 European countries had joined the COMBACTE laboratory network, LAB-Net. By January 2018, 5 years after its launch, the COMBACTE network supported 18 clinical (both observational and intervention) trials on anti-infectives. More than 200 laboratories in COMBACTE LAB-Net are actively involved in these trials.

The capacity and capability of the laboratories to detect CR-GNB, and knowledge on local rates of resistance, are prerequisites for their successful performance in clinical studies. To assess the existing laboratory methods for detection, identification and susceptibility testing of MDR-GNB, a survey was designed and held among European laboratories participating in COMBACTE LAB-Net [10]. The survey represents a descriptive study on distribution and usage of carbapenem resistance detection methods among laboratories in the COMBACTE LAB-Net network in Europe. The purpose of this survey was to select sites for participation in two planned clinical studies: a prospective observational study (EUropean prospective cohort study on Enterobacteriaceae showing REsistance to Carbapenems [EURECA]) and a phase III study (Revisiting serious bacterial infections with innovation [REVISIT]). EURECA (Clintrials.gov NCT02709408) is an observational study, funded by the IMI as part of the COMBACTE-CARE project [10,11]. REVISIT (Clintrials.gov NCT03329092) is a phase III study to evaluate the efficacy, safety and tolerability of aztreonam-avibactam (ATM-AVI) ± metronidazole vs. meropenem ± colistin for the treatment of serious infections due to Gram-negative bacteria, for which there are limited or no treatment options. We hereby summarise the most important findings of the survey.

2. Materials and Methods

2.1. Survey design

The survey questionnaire was designed in 2015 to target detection and identification methods of CR-GNB, susceptibility testing methods (including interpretative criteria), participation in internal and external quality programmes, and detection of specific resistance types. The survey included detailed questions on "in-house" culture and nucleic acid amplification methods used in laboratories for detection of CR-GNB. The survey also addressed sampling strategy, specimen processing, storage, interpretation of results and reporting. The survey also included questions regarding the local rates of carbapenem resistance among *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Acinetobacter* spp. (pathogens targeted in the COMBACTE-CARE observational and phase III studies). Laboratories were also asked to report the total number of the above-mentioned pathogens that they isolated from all sample types during the year preceding the completion of the survey, and the total number of all non-duplicate CR-GNB isolates for the given year. From this information, we calculated the resistance rates.

An online version of the survey was made available in April 2015 and was first piloted in six laboratories in four countries (Belgium, Greece, Italy and Spain) of the COMBACTE LAB-Net network. The questionnaire was amended based on the feedback received from this pilot survey. The final version was used for site selection for the EURECA clinical study.

The questionnaire was later modified and used for preselection of sites for the REVISIT study. The surveys for preselection of sites for the two clinical trials differed only by the study-specific questions and the years of reporting of CR-GNB isolates.

2.2. Data collection and analysis

The questionnaire was sent out to 211 laboratories in 20 European countries between May 2015 and June 2017. Selection of these countries was based on knowledge of their prevalence rates of carbapenem resistance [12,13] and taking into consideration the target countries for the EURECA and REVISIT studies of the sponsor. Data for both studies were collected from 2014 to 2016. Each laboratory was allowed two to three weeks to complete the survey. Completion of surveys was closely monitored and laboratories were reminded to respond within the set deadline via automatic email reminders and, when necessary, via phone calls. Data were analysed using Microsoft Excel 2010 (Microsoft Inc., Redmond, WA).

3. Results

The survey was completed by 165 of 211 (78%) invited laboratories in 20 European countries. Almost half (47%) the respondents (n = 78) were from the Balkan region. Spain (n = 29; 18%), Italy (n = 22; 13%) and Greece (n = 20; 12%) had the most laboratories participate in the survey. The majority of laboratories (n = 113; 68%) served only one hospital. However, 18 (11%) and 21 (13%) laboratories served two or three hospitals, respectively. The remaining laboratories served four or more hospitals (n = 13; 8%).

3.1. Methods used for identification of Gram-negative bacteria and for detection and characterisation of carbapenem-resistant bacteria

3.1.1. Phenotypic methods for detection and identification of CR-GNB

Laboratories used predominantly culture methods to detect CR-GNB directly from clinical samples, but the use of media varied depending on the sample type. Non-selective media were used by 70% to 75% of the laboratories for all samples except rectal swabs. For rectal swabs, 41% of the respondents used non-selective media. Overall, 23% (n=39) of respondents added an antibiotic disc on non-selective media to support CR-GNB detection. Chromogenic media were preferred by between 18% and 24% of respondents, with higher usage for rectal swabs (44%). The predominant identification methods used for Gram-negative bacteria were VITEK (bioMérieux, La Balme-les-Grotte, France) (n=106; 64%) and MALDI-TOF (Bruker Daltonics, Bremen, Germany) (n=81; 49%) (Table 1).

3.1.2. Antimicrobial susceptibility of CR-GNB

Use of the disc diffusion method (n=142; 90%) and minimum inhibitory concentration (MIC) testing (n=142; 90%) was equal to determine the antimicrobial susceptibility of CR-GNB. European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines for interpretation of antimicrobial susceptibility testing were used by 94 laboratories (57%) alone and in combination with Clinical and Laboratory Standards Institute (CLSI) guidelines by another 39 laboratories (24%). CLSI alone was used by 32 laboratories (19%). PCR-based assays to detect genes conferring carbapenem resistance in GNB isolates suspected to be CR were used in half the cases (n=83). MALDI-TOF was used by a handful of laboratories (n=14; 8%) to confirm carbapenem resistance due to β-lactamases among Gram-negative pathogens. The Carba NP [5] test (either in-house or commercial) was used by a total of 47 laboratories (28%). Almost half the laboratories (n=80; 48%) used ertapenem 10 µg discs to screen for carbapenem resistance in Enterobacteriaceae. When asked which carbapenem or combination

Table 1
Available methods for GNB identification and CR detection in participating laboratories.

Available methods for GNB identification and CR detection in participating laboratories	% (n/N)
Molecular diagnostics for direct detection of CR-GNB from clinical samples	
Sputum	7 (12/165)
Endotracheal aspirates	9 (15/165)
Bronchoalveolar lavage	9 (15/165)
Urine	8 (14/165)
Blood cultures	19 (31/165)
Rectal swab	18 (18/98)
Identification methods used for Gram-negative bacteria	
Conventional biochemical tests	63 (105/165)
Matrix Assisted Laser Desorption Ionization - Time of Flight (MALDI-TOF)	49 (81/165)
API system (bioMérieux)	25 (41/165)
Vitek (bioMérieux)	64 (106/165)
Phoenix (Becton Dickinson)	6 (10/165)
MicroScan (Siemens)	17 (29/165)
Breakpoint guidelines used for interpretation of antimicrobial susceptibility testing	
European Committee on Antimicrobial Susceptibility Testing (EUCAST) only	57 (94/165)
Clinical and Laboratory Standards Institute (CLSI) only	19 (32/165)
Both EUCAST and CLSI	24 (39/165)
Antimicrobial susceptibility testing	
Disc diffusion method	90 (142/157)
Minimum inhibitory concentration (MIC) determination	90 (142/157)
Confirmation of carbapenem resistance	
Polymerase chain reaction (PCR)	50 (83/165)
MALDI-TOF	8 (14/165)
(Modified) Hodge test	57 (94/165)
Carbapenemase Nordmann-Poirel (Carba NP) test	
In-house	11 (19/165)
Commercial	17 (28/165)
ROSCO Neo-Rapid CARBA Screen	16 (27/165)
Typing methods for characterisation of carbapenem-resistant GNB	
Pulse-field gel electrophoresis (PFGE)	30 (50/165)
Multi-locus sequence typing (MLST)	22 (36/165)
Repetitive element PCR (rep-PCR)	19 (31/165)
Whole-genome sequencing (WGS)	11 (18/165)
Random amplified polymorphic DNA (RAPD)	8 (13/165)
Enterobacterial repetitive intergenic consensus PCR (ERIC-PCR)*	6 (10/165)
Referring CR-GNB isolates to a specialised laboratory for confirmation	
CR Enterobacteriaceae	47 (77/ 165)
CR *P. aeruginosa*	32 (52/ 165)
CR *Acinetobacter spp.*	30 (50/ 165)

* (for Enterobacteriaceae only) CR - carbapenem-resistant; GNB - Gram-negative bacteria

Table 2
Carbapenems and combination of carbapenems/carbapenemase inhibitors to interpret carbapenem resistance when using the disc diffusion method. The total percentages in each column exceed 100% because laboratories gave more than one answer.

	Enterobacteriaceae	*P. aeruginosa*	*Acinetobacter* spp.
Non-susceptibility to imipenem 10 µg	53% (n = 87)	61% (n = 101)	59% (n = 98)
Non-susceptibility to meropenem 10 µg	65% (n = 107)	65% (n = 108)	63% (n = 104)
Non-susceptibility to ertapenem 10 µg	48% (n = 80)	NA	NA
Non-susceptibility to doripenem 10 µg	8% (n = 13)	9% (n = 15)	10% (n = 16)
Decreased zone to Temocillin 30 µg	19% (n = 31)	NA	NA
Non-susceptibility to carbapenem + boronic acid	31% (n = 51)	15% (n = 24)	13% (n = 21)
Non-susceptibility to carbapenem + EDTA	32% (n = 54)	27% (n = 45)	18% (n = 29)
Non-susceptibility to carbapenem + dipicolinic acid	17% (n = 28)	8% (n = 14)	7% (n = 12)
Non-susceptibility to carbapenem + cloxacillin	18% (n = 30)	8% (n = 13)	7% (n = 12)

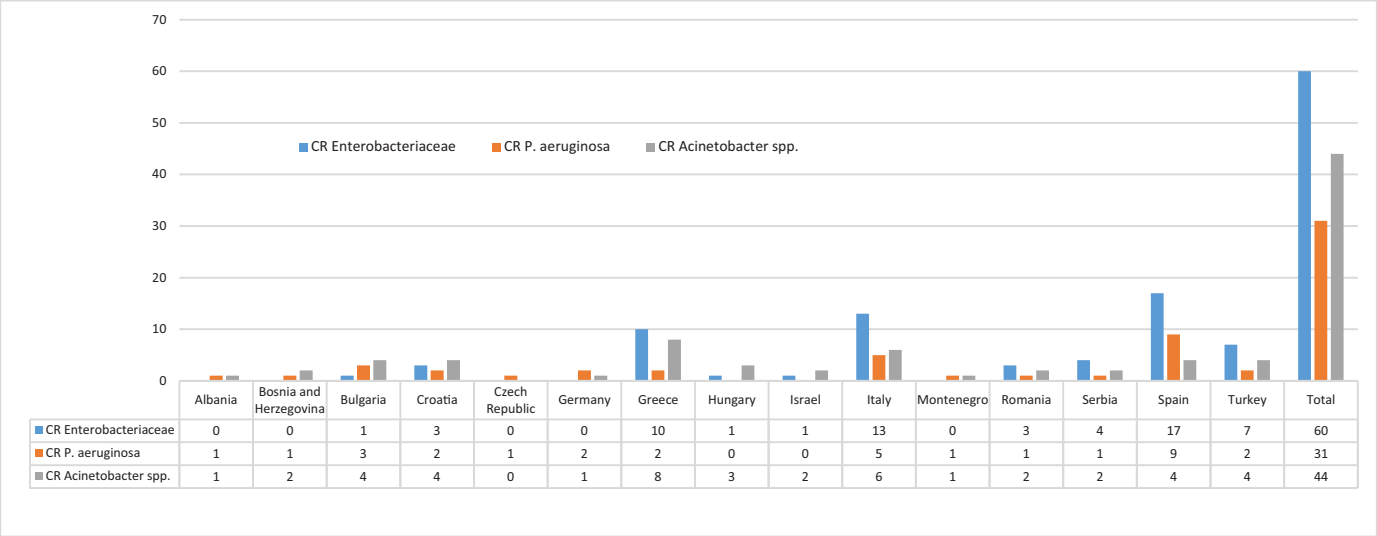
of carbapenem/carbapenemase inhibitor was used for the interpretation of carbapenem resistance by disc diffusion method, 65% of the laboratories (n=107) answered meropenem 10 µg, followed by imipenem 10 µg (n=87; 53%) (Table 2). Similar findings were observed for MIC testing when used to determine non-susceptibility to carbapenems. The survey also showed that boronic acid was the most common inhibitor in phenotypic tests for detecting class A carbapenemase production.

3.1.3. Molecular methods for direct detection and further characterisation of CR-GNB

Molecular methods for direct detection of CR-GNB were less commonly used, and were mainly from blood cultures and rectal swabs (Table 1). The GeneXpert Carba-R BEU instrument (Cepheid, Sunnyvale, USA) was the preferred method in 38 laboratories (23%), followed by in-house endpoint (n=36; 22%) and real-time (n=17; 10%) PCR. Several laboratories characterise the CR-GNB isolates using molecular typing methods, such as pulsed-field gel electrophoresis (PFGE) (n=50; 30%), multi-locus sequence typing (MLST) (n=36; 22%) or even whole-genome sequencing (WGS) (n=18; 11%).

3.1.4. Referral of isolates to a centralised reference laboratory for confirmation

Some of the laboratories reported sending their CR-GNB isolates to a specialised laboratory for further confirmation, and the numbers were as follows: CR Enterobacteriaceae (n=77; 47%), CR *P. aeruginosa* (n=52; 32%) and CR *Acinetobacter spp.* (n=50; 30%).



	Albania	Bosnia and Herzegovina	Bulgaria	Croatia	Czech Republic	Germany	Greece	Hungary	Israel	Italy	Montenegro	Romania	Serbia	Spain	Turkey	Total
CR Enterobacteriaceae	0	0	1	3	0	0	10	1	1	13	0	3	4	17	7	60
CR P. aeruginosa	1	1	3	2	1	2	2	0	0	5	1	1	1	9	2	31
CR Acinetobacter spp.	1	2	4	4	0	1	8	3	2	6	1	2	2	4	4	44

Fig. 1. Number of laboratories that reported outbreaks of CR-GNB by country.

3.2. Patient screening procedures used for carbapenem-resistant Gram-negative bacteria

The majority of laboratories participating in the survey (n=129; 79%) reported analysing samples collected for CR-GNB screening purposes. If CR-GNB had already been isolated from a patient, screening was also performed from body sites other than the infection site in 71% of cases (n=103/145). Intensive care unit (ICU) patients, both ventilated (n=111; 67%) and non-ventilated (n=110; 67%), represented the largest patient population to be screened. Twenty-seven (16%) laboratories reported they also screen patients in oncology/haematology wards. The preferred screening method was culture (n=138; 84%). Rapid diagnostic tests were also used, with GeneXpert Carba-R BEU the most widely used method (n=22; 14%).

3.3. Accreditation and participation in surveillance and external quality assessment programmes

More than half the laboratories reported that they were accredited (n=90; 55%) and the ISO 15189 standard was used for that in 82 (50%) laboratories. Sixty-nine of 98 (70%) laboratories participated in national programmes for external quality control. Less than half the respondents (n=44/98; 45%) participated in international surveillance programmes, such as CAESAR, EARS-Net, SENTRY, SMART and T.E.S.T.

3.4. Local prevalence of CR-GNB

Sixty of the 165 (36%) laboratories reported an outbreak of CR *Enterobacteriaceae* during one of the two years preceding the completion of the survey (Fig. 1). The percentage was lower for CR *P. aeruginosa* (19%; n=31) and CR *Acinetobacter* spp. (27%; n=44) outbreaks.

The majority of participating laboratories (n=98) reported levels of CR *E. coli* isolates between 1% and 10% per year (within the period of 2014-2016) (Table 3). Fifty-eight of 165 (37%) laboratories did not isolate CR *E. coli* during the survey. Only three laboratories, in Albania (n=1), Greece (n=1) and Turkey (n=1), reported more than 10% CR *E. coli.*

The levels of CR *K. pneumoniae* were substantially higher than these of CR *E. coli*. Only 12 laboratories (8%) reported no isolation of CR *K. pneumoniae* during the survey. The majority of laboratories reported between 1% to 10% carbapenem resistance. Greece was the country with most laboratories (n=17) that reported CR rates above 25%.

Most of the laboratories reported rates of CR *P. aeruginosa* between 11% and 25% or between 26% and 50%. The laboratories that reported these findings were predominantly situated in Greece, Italy and Spain.

High rates of *CR Acinetobacter* spp. above 50% were reported by 74 laboratories (47%) during the period of the survey. More than half these laboratories were in Greece (n=12), Italy (n=9), Romania (n=7) and Turkey (n=11). Laboratories from other Balkan countries also reported high rates of CR *Acinetobacter* spp. All laboratories from Bosnia and Herzegovina and almost all respondents from Croatia reported >50% CR *Acinetobacter* spp. isolates per year. Overall, all participating countries in the Balkan region (except Albania) had one of the highest rates of CR Acinetobacter spp. >50% (Table 4).

4. Discussion

This survey on capacity and capability of the laboratories to detect CR-GNB, and local rates of resistance, was designed to help us select the most appropriate sites for participation in two clinical studies, EURECA and REVISIT. The survey was not designed to collect surveillance data on antibiotic resistance among GNB in Europe. Moreover, there was selection bias as targeted laboratories were preselected to participate in the survey based on information from other studies within the COMBACTE network. Most of the laboratories were in tertiary hospitals and are highly performing and well-equipped to detect CR-GNB (i.e. using MLST or PFGE to further characterise these isolates). Nevertheless, we revealed some interesting findings in the COMBACTE network on carbapenem resistance in previously underreported regions in Europe. As expected, and in line with previous reports [12-14], laboratories from Greece, Italy and Spain reported high prevalence rates of CR pathogens. The majority of laboratories from Romania also reported high rates of CR *K. pneumoniae* (11-25%) and *P. aeruginosa* (26-50%). Strikingly, of all GNB included in the survey, the rates of CR *Acinetobacter* spp. were the highest, with many laboratories from almost all countries reporting resistance above 50%. Similar findings were also reported by the European Center for Disease Prevention and Control (ECDC) reflecting only invasive CR-GNB isolates in EU countries [13]. Data

Table 3
Number of laboratories that reported various rates (0%; 1-10%; 11-25%; 26-50%; >50%) of carbapenem resistance in *E. coli, K. pneumoniae, P. aeruginosa* and *Acinetobacter* spp. throughout the survey period of 2014-2016. Some of the numbers do not add up to 165 (total number of respondents) because some of the laboratories did not provide prevalence data for all or some of the four pathogen groups. However, these laboratories did answer the remaining questions in the survey.

	CR *E. coli*			CR *K. pneumoniae*					CR *P. aeruginosa*					CR *Acinetobacter* spp.				
	0%	1-10%	11-25%	0%	1-10%	11-25%	26-50%	>50%	0	1-10%	11-25%	26-50%	>50%	0	1-10%	11-25%	26-50%	>50%
Albania	1	0	1	1	0	0	0	1	1	0	0	1	0	1	0	0	0	1
Bosnia and Herzegovina	3	1	0	3	1	0	0	0	1	0	3	0	0	0	0	0	0	4
Bulgaria	5	3	0	2	4	1	1	0	1	2	2	3	0	0	2	2	0	4
Croatia	3	3	0	0	5	0	1	0	0	1	4	1	0	0	0	0	1	5
Czech Republic	3	2	0	2	3	0	0	0	2	0	3	0	0	2	3	0	0	0
France	0	2	0	0	2	0	0	0	0	0	0	2	0	0	1	1	0	0
Germany	1	8	0	0	9	0	0	0	0	4	5	0	0	0	7	2	0	0
Greece	7	12	1	0	2	1	12	5	0	3	6	10	1	0	1	1	6	12
Hungary	8	0	0	2	6	0	0	0	0	2	2	4	0	0	0	3	0	5
Israel	2	5	0	0	7	0	0	0	1	2	2	2	1	1	0	1	1	4
Italy	7	15	0	0	9	7	5	1	0	8	11	2	0	0	4	2	7	9
Kosovo	1	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1
Poland	1	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0
Romania	3	6	0	0	3	4	2	0	0	0	2	5	2	0	0	2	0	7
Serbia	5	8	0	0	9	4	0	0	0	3	6	4	0	0	3	1	2	6
Spain	8	21	0	1	23	5	0	0	0	11	11	6	0	4	5	8	7	5
Switzerland	0	1	0	0	1	0	0	0	1	0	0	0	0	0	1	0	0	0
Turkey	0	11	1	0	1	8	3	0	0	0	3	7	2	0	1	0	1	11
Total	**59**	**98**	**3**	**13**	**86**	**30**	**24**	**7**	**7**	**36**	**62**	**48**	**6**	**9**	**28**	**23**	**26**	**74**

Table 4
Rates of carbapenem-resistant *E. coli, K. pneumoniae, P. aeruginosa* and *Acinetobacter* spp. by country. The number of laboratories per country is given in brackets after the country name. The total number of laboratories is less than 165 because some of the laboratories did not provide prevalence data for all or some of the four groups of pathogens.

Country	CR *E. coli*(%)	CR *K. pneumoniae*(%)	CR *P. aeruginosa*(%)	CR *Acinetobacter* spp.(%)
Albania (2)	7.02	28.72	15.18	37.50
Bosnia and Herzegovina (4)	0.04	0.25	11.88	80.38
Bulgaria (8)	0.77	6.40	18.31	53.50
Croatia (6)	0.10	9.29	19.64	82.08
Czech Republic (5)	0.02	0.95	11.07	2.46
France (2)	0.51	4.11	30.12	11.19
Germany (9)	0.09	0.49	11.63	6.14
Greece (20)	1.66	33.39	29.52	65.90
Hungary (8)	0.00	0.93	24.51	51.65
Israel (7)	0.14	2.57	11.82	43.06
Italy (22)	0.29	17.14	15.80	47.65
Kosovo (1)	0.00	0.00	17.20	79.08
Poland (1)	0.00	0.23	11.81	30.56
Romania (9)	0.22	16.22	38.23	66.20
Serbia (13)	0.57	10.53	22.71	74.68
Spain (29)	0.13	4.90	14.02	24.04
Switzerland (1)	0.02	0.48	0.00	3.23
Turkey (12)	2.98	20.53	35.98	81.50

collected in the survey confirm this trend and shed more light on the Western Balkan countries, for which published data on CR *Acinetobacter* spp. in the literature are scarce [15]. Almost all laboratories from this area (Albania, Bosnia and Herzegovina, Croatia and Serbia) reported extremely high rates of CR *Acinetobacter* spp., sometimes higher than 90%. This is an alarming trend that should be closely monitored and reversed by taking appropriate local infection control measures [16,17].

As expected, the majority of laboratories relied mostly on culture methods to detect these pathogens directly from clinical samples. Rapid diagnostic tests, such as GeneXpert Carba-R BEU, were used routinely in 23% of the participating laboratories. Almost two-thirds of the participating laboratories indicated they rely on the interpretation of meropenem susceptibility testing to detect carbapenem resistance by the disc diffusion method. A total of 48% of the respondents also used ertapenem to screen for carbapenem resistance in Enterobacteriaceae. Ertapenem has been shown to be a good screening tool in populations with a high prevalence of carbapenemases [18]. Previous studies [19] have shown that meropenem and ertapenem performed better than imipenem in the distinction between KPC- and VIM-producers and wild-type strains. The EUCAST guidelines for interpretation of antimicrobial susceptibility testing (AST) results were used most frequently in the laboratories participating in our survey. A total of 32 laboratories (19%), predominantly from Greece (n=10; 50% of the Greek laboratories in the survey) reported using only the CLSI guidelines. Another 40% reported using CLSI guidelines in combination with EUCAST. This is in line with the observation of the EUCAST committee in January 2018 regarding implementation of EUCAST breakpoints [20]. Also, in Greece less than 10% of the laboratories adopted the EUCAST disc diffusion method. Regardless of the guidelines used (EUCAST or CLSI) [21,22], the AST methodologies for detection of CR-GNB do not differ substantially, except for the difference in breakpoints for carbapenems. Although the current EUCAST clinical susceptible breakpoints for imipenem and meropenem (≤2 mg/L) are higher than those suggested by CLSI, they are supported by pharmacodynamic data and clinical study data [23], with no evidence of poorer outcomes in patients treated with carbapenems for infections caused by carbapenemase producers with MICs ≤4 mg/L.

Surprisingly, 8% (n=14) of the laboratories, among which four were from the Czech Republic, two were from Germany and three were from Spain, used MALDI-TOF not only as a method for identification of microorganisms, but also as a tool to confirm the presence of carbapenem resistance due to β-lactamases among Gram-negative bacteria. Previous studies [24-26] have shown that it is a convenient and rapid method that takes 3 h to detect carbapenemases and that is comparable to the Carba NP test. Hence, once used by the laboratories for identification, it can also serve to speed up the detection of CR-GNB. However, MALDI-TOF false-negative results with OXA-48-type producers and the need for an additional buffer might make the test less attractive, particularly in countries with higher prevalence of OXA-48-producing Enterobacteriaceae. Increased availability of MALDI-TOF for identification in microbiology laboratories could potentially increase its use for CR confirmation.

5. Conclusion

This survey enabled us to identify the methods used locally for the detection of CR-GNB and to assess resistance rates of GNB to carbapenems. Sites in the Balkan countries (Bosnia and Herzegovina, Croatia, Greece, Romania and Serbia), as well as Italy and Spain had the highest prevalence of CR-GNB isolates. Based on these results, we selected sites with high prevalence of CR-GNB for the EURECA clinical study and the sites subsequently successfully recruited many patients in this study (www.combacte.com). Since the start of the recruitment period in May 2016, several sites in Greece, Spain and Serbia enrolled more than 100 patients each. The achievements of these sites in the EURECA study underscores the importance of collecting recent antibiotic resistance rates for the purposes of site selection in clinical trials. Furthermore, an ongoing collection of real-time data for site selection would significantly shorten the preparatory phase of clinical trials. The ultimate goal of LAB-Net and COMBACTE is to create a self-sustainable network to conduct clinical trials where such site selection data will be available in real time, which will help in selecting the best sites for patient enrolment and shorten the duration of clinical trials. This is crucial given the complexity of anti-infective clinical trials and their importance in the increasing need to boost clinical development of new anti-infectives.

We conclude that a survey for medical microbiology laboratories adds to the efficiency of the site selection process for clinical trials in infectious diseases. Site selection gives us a certain level of confidence that the chosen laboratories are capable of identifying the pathogens of interest and are well prepared to engage in a clinical trial. The experience within the COMBACTE projects, and in particular COMBACTE-CARE, has shown that microbiology laboratories play an indispensable role in anti-infective clinical studies and their performance is vital for the successful outcome of clinical trials.

Acknowledgements

We would like to thank all laboratories in the COMBACTE LAB-Net network that completed the survey.

Funding

The research leading to these results has received support from the Innovative Medicines Initiative Joint Undertaking through the COMBACTE-NET (Grant agreement No. 115 523) and COMBACTE-CARE (Grant agreement No. 115 620) projects, resources of which are composed of financial contribution from the European Union's Seventh Framework Programme (FP7/2007-2013) and EFPIA companies' in kind contribution.

Competing interests

None to declare.

Ethical approval

Not required.

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