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Enterobacterales carrying chromosomal AmpC β-lactamases in Europe (EuESCPM): Epidemiology and antimicrobial resistance burden from a cohort of 27 hospitals, 2020–2022

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ABSTRACT

Introduction: The ESCPM group (*Enterobacter* species including *Klebsiella aerogenes* - formerly *Enterobacter aerogenes*, *Serratia* species, *Citrobacter freundii* complex, *Providencia* species and *Morganella morganii*) has not yet been incorporated into systematic surveillance programs.
Methods: We conducted a multicentre retrospective observational study analysing all ESCPM strains isolated from blood cultures in 27 European hospitals over a 3-year period (2020–2022). Diagnostic approach, epidemiology, and antimicrobial susceptibility were investigated.
Results: Our study comprised 6,774 ESCPM isolates. MALDI-TOF coupled to mass spectrometry was the predominant technique for bacterial identification. Susceptibility to new β-lactam/β-lactamase inhibitor combinations and confirmation of AmpC overproduction were routinely tested in 33.3% and 29.6% of the centres, respectively. The most prevalent species were *E. cloacae* complex (44.8%) and *S. marcescens* (22.7%). Overall, third-generation cephalosporins (3GC), combined third- and fourth-generation cephalosporins (3GC + 4GC) and carbapenems resistance phenotypes were observed in 15.7%, 4.6%, and 9.5% of the isolates, respectively. AmpC overproduction was the most prevalent resistance mechanism detected (15.8%). Among carbapenemase-producers, carbapenemase type was provided in 44.4% of the isolates, VIM- (22.9%) and OXA-48-enzyme (16%) being the most frequently detected. *E. cloacae* complex, *K. aerogenes* and *Providencia* species exhibited the most notable cumulative antimicrobial resistance profiles, with the former displaying 3GC, combined 3GC + 4GC and carbapenems resistance phenotypes in 15.2%, 7.4%, and 12.8% of the isolates, respectively. *K. aerogenes* showed the highest rate of both 3GC resistant phenotype (29.8%) and AmpC overproduction (32.1%), while *Providencia* species those of both carbapenems resistance phenotype (42.7%) and carbapenemase production (29.4%). ESCPM isolates exhibiting both 3GC and combined 3GC + 4GC resistance phenotypes displayed high susceptibility to ceftazidime/avibactam (98.2% and 95.7%, respectively) and colistin (90.3% and 90.7%, respectively). Colistin emerged as the most active drug against ESCPM species (except those intrinsically resistant) displaying both carbapenems resistance phenotype (85.8%) and carbapenemase production (97.8%).
Conclusions: This study presented a current analysis of ESCPM species epidemiology in Europe, providing insights to inform current antibiotic treatments and guide strategies for antimicrobial stewardship and diagnostics.

1. Introduction

Several reports have highlighted how the SARS-CoV-2 pandemic has exacerbated a long-standing antimicrobial resistance crisis, setting back progress and challenging planned mitigation efforts [1,2]. The overreliance on empirical antibiotic use, even in the face of a low prevalence of bacterial coinfections and superinfections in COVID-19 patients, constant hospital reorganizations to accommodate COVID-19 cases, and disruptions to antimicrobial stewardship programs emerged as the primary drivers behind the surge in antimicrobial resistance during the pandemic [3]. The response to this resurgence in antimicrobial resistance demanded a transnational approach with actions at various levels, including a critical focus on strengthening antimicrobial resistance surveillance [2]. In Europe, the European Antimicrobial Resistance Surveillance Network (EARS-Net) plays a pivotal role in monitoring the antimicrobial susceptibility of eight bacterial pathogens commonly associ-

ated with human infections, which include *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter species, Streptococcus pneumoniae, Staphylococcus aureus, Enterococcus faecalis*, and *Enterococcus faecium* [4]. Within *Enterobacterales*, the ESCPM species (*Enterobacter* species including *Klebsiella aerogenes* - formerly *Enterobacter aerogenes, Serratia* species, *Citrobacter freundii* complex, *Providencia* species and *Morganella morganii*) are notably absent from systematic surveillance programs, despite their involvement in a wide spectrum of community- and healthcare-associated infections. However, understanding their antimicrobial resistance mechanisms is recognised as imperative [5]. Indeed, ESCPM species harbour chromosomally-encoded inducible AmpC β-lactamases and can easily segregate stably de-repressed mutants able to overexpress these β-lactamases and hydrolyse multiple antibiotics [5–7]. The optimal approach to treat infections caused by these pathogens remains a subject of ongoing debate [8,9], with carbapenems and cefepime emerging as the most frequently recommended options [5,10–16]. Furthermore, multiple resistance mechanisms can coexist, including the production of β-lactamases (such as extended-spectrum β-lactamases - ESBLs and carbapenemases) and compromised outer membrane permeability [17]. Notably, *Enterobacter* species featured among the top three species displaying reduced carbapenem susceptibility in a French epidemiological study based on 2012–2014 data [18]. Additionally, a recent global surveillance program revealed an increasing prevalence of metallo β-lactamase (MBL) producers among meropenem-non-susceptible *Enterobacter* species and *Citrobacter* species isolates [19–21]. Furthermore, antibiotic prescribing practices and the resulting selective pressure on bacteria may have influenced epidemiological shifts. This was evidenced in studies where the use of ceftazidime/avibactam may have contributed to the emergence of MBL producers in *Enterobacterales* [22]. The utilization of third-generation cephalosporins (3GC) also poses a non-negligible risk for resistance development, particularly in *Enterobacter* species, *K. aerogenes*, and *C. freundii* complex [7,16]. Recognizing the challenges in reporting antimicrobial susceptibility and treating ESCPM infections, our study sought to provide insights to address the issue of antimicrobial resistance in Europe. We investigated the epidemiology and antimicrobial susceptibility of ESCPM organisms isolated from blood cultures (BCs) during the initial three years of the SARS-CoV-2 pandemic in a large cohort of European hospitals.

2. Methods

2.1. Study design

We conducted a multicentre retrospective observational study, encompassing all consecutive ESCPM species isolates detected from BCs of hospitalised patients in 27 European hospitals across 14 countries, with a cumulative bed capacity of 35 000 (Figure 1). Data were collected between 1 January, 2020, and 31 December, 2022. Duplicate isolates obtained within a 20-day interval from the same patient and with the same antibiotic susceptibility testing results were considered as part of a single positive BC episode and thus excluded from the analysis.

2.2. Survey on the diagnostic approach to ESCPM species

We conducted a survey to assess microbiological diagnostic practices for ESCPM species in the European centres participating in the study. The study coordinating centre designed a questionnaire, which was distributed to all laboratories involved. The questionnaire comprised 21 questions, categorised into 2 sections (refer to Tables S1 and S2). These questions covered various aspects, including the type of centre (e.g., hospital type, number of hospital beds), laboratory activities (such as the number of ESCPM species isolates tested and the methods used for antimicrobial susceptibility testing), and technical details like the antimicrobial agents routinely tested for ESCPM species and the screening methods for 3GC and/or combined third- and fourth-generation cephalosporins (3GC + 4GC) and/or carbapenem-resistant ESCPM species isolates.

2.3. ESCPM species identification and susceptibility testing

For each ESCPM species-positive BC episode, we documented the clinical setting in which the pathogen was isolated, including emergency, medical, surgical, COVID-19 wards, ICU, and COVID-19 ICU. We recorded the results of susceptibility testing along with the species identification method and the clinical breakpoints used by each institution during the study period. Antimicrobial susceptibility testing results (MICs or inhibition zone diameters) were interpreted in accordance with the guidelines provided by the European Committee on Antimicrobial Susceptibility Testing (EUCAST, version 13.1) and the Clinical & Laboratory Standards Institute (CLSI, M100Ed33: 2023 Performance Standards for Antimicrobial Susceptibility Testing) [23,24]. These guidelines were also applied to identify ESBL-producing, AmpC overproducing-, and carbapenemase-producing ESCPM strains, with confirmatory tests for resistance mechanisms performed once the conventional antimicrobial susceptibility testing results became available.

2.4. Definitions

We defined the following susceptibility patterns based on EUCAST v. 13.1 breakpoints. 3GC susceptibility phenotype was defined as a susceptibility pattern characterised by susceptibility to 3GC (cefotaxime or ceftriaxone and ceftazidime), cefepime, and carbapenems (ertapenem, imipenem, and meropenem). 3GC resistance phenotype was defined as a susceptibility pattern characterised by resistance to at least one antimicrobial agent among cefotaxime or ceftriaxone and ceftazidime and susceptibility to cefepime and carbapenems (ertapenem, imipenem, and meropenem). Combined 3GC + 4GC resistance phenotype was defined as a susceptibility pattern characterised by resistance to cefepime, at least one antimicrobial agent among cefotaxime or ceftriaxone and ceftazidime and susceptibility to carbapenems (ertapenem, imipenem, and meropenem). Carbapenems resistance phenotype was defined as a susceptibility pattern characterised by resistance to at least one antimicrobial agent among ertapenem, imipenem, and meropenem. Susceptibility to aminoglycosides was defined as a susceptibility pattern characterised by susceptibility to at least one antimicrobial agent among gentamicin and amikacin. Susceptibility to fluoroquinolones was defined as a susceptibility pattern characterised by susceptibility to either ciprofloxacin or both ciprofloxacin and levofloxacin.

2.5. Statistics

We presented descriptive data using absolute counts (n) and relative percentages (%) for categorical variables. Summary statistics for MIC values included the MIC_{50} and MIC_{90}. Summary statistics for inhibition zone diameters included range and median. Data analysis was performed using Microsoft Excel (Office 365) and Python 3.10.

3. Results

3.1. Diagnostic approach to ESCPM species around Europe

ESCPM species identification was primarily conducted using Vitek-2 (bioMérieux, Marcy l'Étoile, France) for biochemical identification, Vitek MS (bioMérieux, Marcy l'Étoile, France), or Bruker

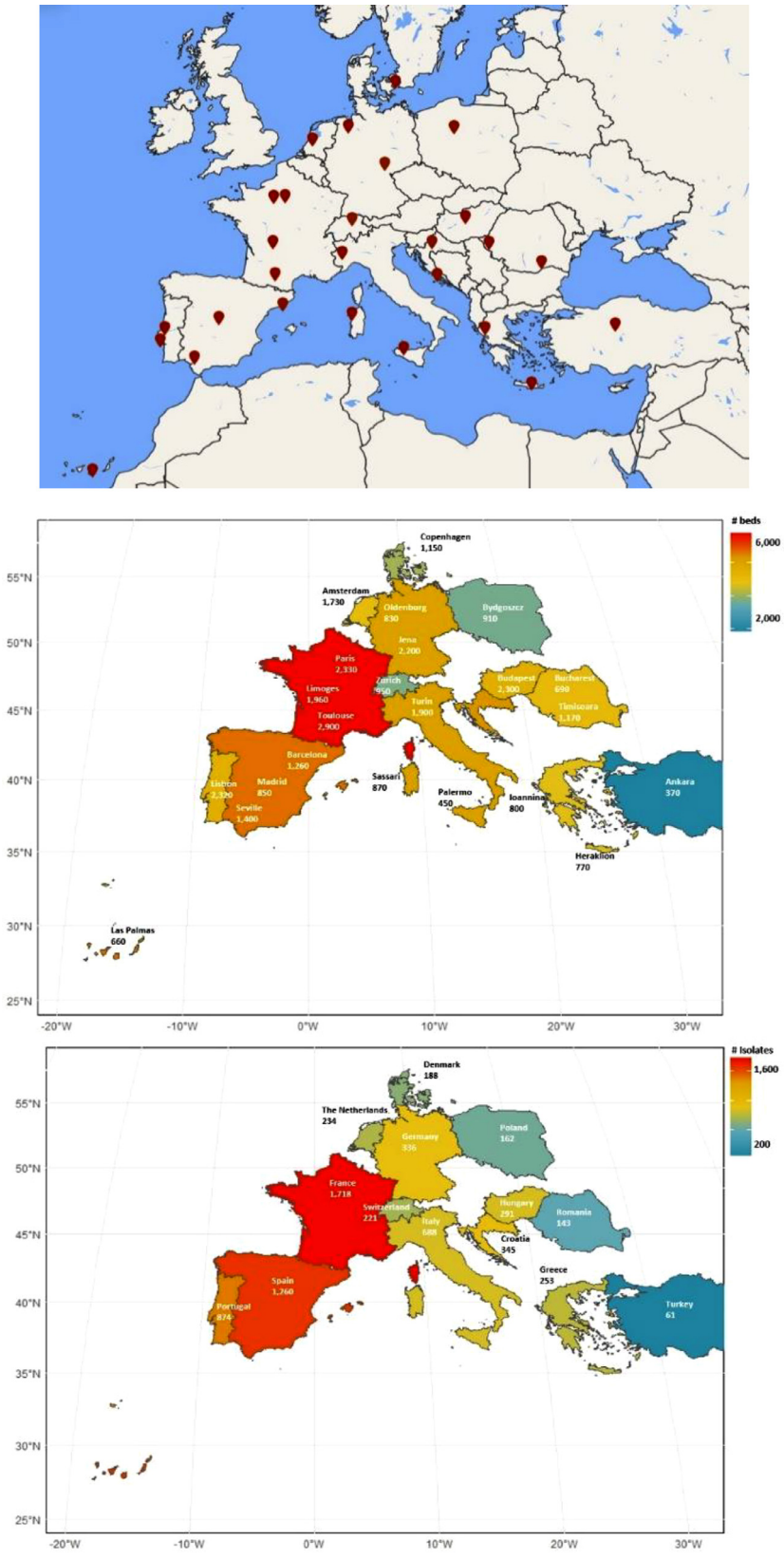

Fig. 1. EuESCPM collaborative centres: geography, bed capacity, and number of isolates.

Biotyper (Bruker Daltonics GmbH, Bremen, Germany) for MALDI-TOF mass spectrometry-based identification (refer to Table S1). Susceptibility testing results were obtained through various methods, including broth microdilution commercial systems (Vitek-2, bioMérieux, Marcy l'Étoile, France; Microscan WalkAway 96 Plus, Beckman Coulter, Switzerland; BD Phoenix™ Becton Dickinson, USA), gradient diffusion strip method (Etest, bioMérieux, Marcy l'Étoile, France), and disk diffusion, following the recommendations provided by the respective manufacturers.

Regarding antibiotic activity on ESCPM species, most centres reported routinely testing susceptibility to 3GC (100%), cefepime (81.5%), and fosfomycin (88.9%), while susceptibility to new β-lactam/β-lactamase inhibitor combinations such as ceftolozane/tazobactam and ceftazidime/avibactam was carried out by 33.3% of them (Table S2). Fosfomycin susceptibility test was predominantly carried out using automated broth microdilution (62.5%) while colistin testing was primarily conducted using manual broth microdilution (87%) with various kits (UMIC Colistin kit, Biocentric, Bandol, France; MICRONAUT MIC-Strip colistin, MERLIN Diagnostika Gmbh, Bornheim-Hersel, Germany; ComASP Colistin, Liofilchem, Roseto degli Abbruzzi, Italy). Testing for ESBL production based on the in vitro inhibition of ESBL activity by clavulanic acid was carried out in 77.7% of the centres, mainly by a phenotypic test alone (74.1%) including combination disk test or double-disk synergy test (65%) and automated broth microdilution (35%). Testing for AmpC overproduction was routinely performed in 29.6% of the centres using cloxacillin supplemented agar (bioMérieux). Testing for carbapenemase production was carried out in 96.3% of the centres, mainly by a phenotypic followed by genotypic test (44.4%) or a phenotypic test alone (40.7%) as the lateral flow immunochromatographic assays (63.6%) targeting the main carbapenemase enzymes KPC, NDM, VIM, IMP and OXA-48-like (RESIST-5 O.O.K.N.V, Coris Bioconcept, Gembloux, Belgium and NG-test Carba 5, NG Biotech, Guipry, France).

3.2. Epidemiology of ESCPM species isolated from positive blood cultures

In this study, 6774 ESCPM isolates met the inclusion criteria (see Table 1). Almost half of these isolates (43.9%) were identified in centres from France and Spain (refer to Table S3). The most prevalent species were *E. cloacae* complex (44.8%) and *S. marcescens* (22.7%) (refer to Table 1). When comparing countries, the most frequently isolated species varied (Table S3). Overall, ESCPM species were predominantly identified in patients admitted to medical wards (37.2%) and ICUs (30.9%) (Figure 2). Some centres provided data on ESCPM species identification in patients with COVID-19 admitted to dedicated wards (refer to Figure S1). This analysis of 3656 ESCPM isolates revealed that among COVID-19 patients, the most frequently encountered species were *E. cloacae* complex, *K. aerogenes*, and *S. marcescens* in COVID-19 ICUs, and *E. cloacae* complex and *S. marcescens* in COVID-19 wards.

3.3. Burden of antimicrobial resistance in ESCPM species

Detailed susceptibility testing results were shown in Table S4. Overall, ESCPM species were highly susceptible to cefepime (MIC_{50}–MIC_{90} 1–4 mg/L; EUCAST 87.3%; CLSI 92.3%), ceftazidime/avibactam (MIC_{50}–MIC_{90} 2–8 mg/L; EUCAST 90.4%; CLSI 85.6%), ertapenem (MIC_{50}–MIC_{90} 0.19–0.5 mg/L; EUCAST 90.6%; CLSI 95.5%), imipenem (MIC_{50}–MIC_{90} 1–2 mg/L; EUCAST 95.6%; CLSI 93.6%), meropenem (MIC_{50}–MIC_{90} 0.25–0.25 mg/L; EUCAST 97.3%; CLSI 96.4%), colistin (MIC_{50}–MIC_{90} 1–2 mg/L; EUCAST 95.4%; CLSI 95.6%), gentamicin (MIC_{50}–MIC_{90} 2–2 mg/L; EUCAST 91%; CLSI 93.4%), and amikacin (MIC_{50}–MIC_{90} 2–8 mg/L; EUCAST

Table 1
Distribution of ESCPM species positive blood culture episodes.

Year	ESCPM species positive BC episode, n	*Enterobacter cloacae* complex[1] % (n)	*Klebsiella aerogenes* % (n)	*Enterobacter* non-*cloacae* complex[2] % (n)	*Serratia marcescens* % (n)	*Citrobacter freundii* complex[3] % (n)	*Providencia* species % (n)	*Morganella morganii* % (n)
2020	2080	44.5 (926)	14.2 (296)	2.7 (57)	21.7 (452)	6.1 (126)	2.6 (53)	8.2 (170)
2021	2402	44.3 (1062)	12.8 (307)	2.1 (51)	24.2 (582)	5.5 (133)	2.3 (55)	8.8 (212)
2022	2292	45.7 (1048)	12.9 (295)	2.4 (55)	22 (503)	6.4 (146)	2.7 (63)	7.9 (182)
Total	6774	44.8 (3036)	13.3 (898)	2.4 (163)	22.7 (1537)	6 (405)	2.5 (171)	8.3 (564)

[1] *Enterobacter cloacae* complex corresponds to *E. cloacae*, *E. asburiae*, *E. hormaechei*, *E. kobei*, *E. ludwigii*, *E. mori*, and *E. nimipressuralis*.
[2] *Enterobacter* non-*cloacae* complex corresponds to *Enterobacter* species not included in the *Enterobacter cloacae* complex.
[3] *Citrobacter freundii* complex corresponds to *C. freundii*, *C. braakii*, *C. youngae*, *C. portucalentis*, *C.gillenii*, *C.murliniae*, *C. sedlakii* and *C. wekmenii*.Abbreviations: BC, blood culture.

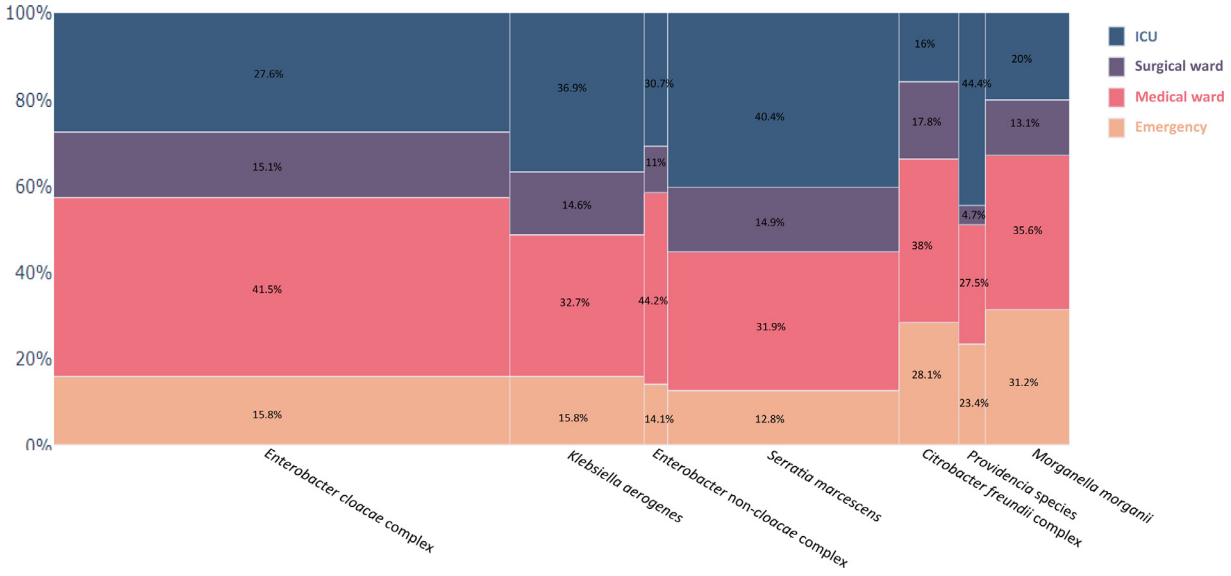

Fig. 2. Distribution of ESCPM species according to hospital ward (Marimekko plot: the width of each column represents the proportion of the ESCPM species within the entire collection. The height of each bar represents the proportion of each ESCPM species within each hospital ward).

96%; CLSI 96.8%). Among *E. cloacae* complex isolates, over 90% susceptibility was achieved by imipenem (MIC_{50}–MIC_{90} 0.5–2 mg/L; EUCAST 96.5%; CLSI 96.2%), meropenem (MIC_{50}–MIC_{90} 0.25–0.25 mg/L; EUCAST 97.2%; CLSI 96.3%), colistin (MIC_{50}–MIC_{90} 0.5–2 mg/L; EUCAST 94.3%; CLSI 94.5%), and amikacin (MIC_{50}–MIC_{90} 2–8 mg/L; EUCAST 96.5%; CLSI 96.9%). *Providencia* species showed the most resistant cumulative antimicrobial susceptibility profile, with higher percentages for most antimicrobials tested. Of note, 31% of *Providencia* species isolates were detected in a Romanian centre and characterised as carbapenemase-producers. The most active in vitro antimicrobials against *Providencia* species isolates were ertapenem (MIC_{50}–MIC_{90} 0.5–8 mg/L; EUCAST 69.1%; CLSI 70.4%), meropenem (MIC_{50}–MIC_{90} 0.5–16 mg/L; EUCAST 70.2%; CLSI 65.1%), and amikacin (MIC_{50}–MIC_{90} 8–64 mg/L; EUCAST 60.5%; CLSI 59.6%). Of note, among all ESCPM isolates, high susceptibility rates to cefepime were observed in *K. aerogenes* (MIC_{50}–MIC_{90} 1–8 mg/L; EUCAST 90.6%; CLSI 95.8%), *S. marcescens* (MIC_{50}–MIC_{90} 1–1 mg/L; EUCAST 96.8%; CLSI 98.5%), *C. freundii* complex (MIC_{50}–MIC_{90} 1–1 mg/L; EUCAST 93.4%; CLSI 96%) and *M. morganii* (MIC_{50}–MIC_{90} 1–1 mg/L; EUCAST 97%; CLSI 98.1%) while lower values were observed in *E. cloacae* complex (MIC_{50}–MIC_{90} 1–8 mg/L; EUCAST 82.3%; CLSI 88%) and *Providencia* species (MIC_{50}–MIC_{90} 8–64 mg/L; EUCAST 57.1%; CLSI 62.1%). Likewise, over 90% susceptibility rates to piperacillin/tazobactam were observed in *S. marcescens* (MIC_{50}–MIC_{90} 4–8 mg/L; EUCAST 92.3%; CLSI 95.5%) and *M. morganii* (MIC_{50}–MIC_{90} 4–8 mg/L; EUCAST 95%; CLSI 97%).

Overall, ESCPM species displayed 3GC, combined 3GC + 4GC, and carbapenems resistance phenotypes in 15.7%, 4.6%, and 9.5% of the isolates, respectively (Table 2). ESBL expression, AmpC overproduction, and carbapenemase production were detected in 6.4%, 15.8%, and 3.1% of the isolates, respectively. Among ESCPM species displaying carbapenems resistance phenotypes, 70.1% ($n = 451/643$) was non-carbapenemase-producer (data not shown). Among carbapenemase-producers, the laboratories involved in the project did not provide the type of enzyme involved in the resistance mechanism in the majority of the cases (55.6%, $n = 114/205$). When information was available ($n = 91/205$), the most frequent were VIM- (22.9%, $n = 47$) and OXA-48- enzymes (16%, $n = 33$). KPC-, NDM-, IMI-, and multiple-carbapenemases-producers were very rarely identified (1.5%, 2.5%, 0.5%, and 1%, respectively). *E. cloacae* complex displayed ESBL production, AmpC overproduction and carbapenemase production in 11.4%, 17.1%, and 3.5% of the isolates, respectively. *K. aerogenes* showed the highest rate of both 3GC resistant phenotype (29.8%) and AmpC overproduction (32.1%), while *Providencia* species those of both carbapenems resistance phenotype (42.7%) and carbapenemase production (29.4%).

Among ESCPM isolates exhibiting 3GC susceptibility phenotype, over 90% activity was shown by piperacillin/tazobactam (98.1%), ceftolozane/tazobactam (99.6%), ceftazidime/avibactam (100%), colistin (93.4%), aminoglycosides (98%), fluoroquinolones (95.9%), and sulfamethoxazole/trimethoprim (93.7%) (Table 3). Among those exhibiting 3GC resistance phenotype, over 90% activity was observed for ceftazidime/avibactam (98.2%), colistin (90.3%) as well as cefepime (100%) by definition. The most active drugs against ESCPM isolates exhibiting combined 3GC + 4GC resistance phenotype were ceftazidime/avibactam (95.7%) and colistin (90.7%) as well as carbapenems (100%) by definition, whereas susceptibility to aminoglycosides, fluoroquinolones, and sulfamethoxazole/trimethoprim (44.3%, 32.9%, and 36.4%, respectively) significantly decreased in comparison with both 3GC susceptible (98%, 95.9%, and 93.7%, respectively) and resistant strains (89.6%, 87.1%, and 84.9%, respectively). Among ESCPM isolates exhibiting carbapenems resistance phenotype, the most active drugs were colistin (85.8%) followed by ceftazidime/avibactam (66%) and aminoglycosides (65.8%). These agents showed higher activity against strains displaying carbapenems resistance phenotype due to a mechanism other than the production of carbapenemase (92.9%, 82.8% and 91.1%, respectively). Among ESBL-producers, the most active drugs were carbapenems (99.2%), ceftazidime/avibactam (94.3%), and colistin (93%), whereas susceptibility rates to cefepime, piperacillin/tazobactam, and ceftolozane/tazobactam were 20.9%, 32.2%, and 58.3%, respectively. Among AmpC overproducers, over 90% activity was shown by carbapenems (99.2%), ceftazidime/avibactam (98.1%), and colistin (93.8%), whereas significantly higher activity in comparison with those observed against ESBL-producers was shown by cefepime (84.4% vs. 20.9%), aminoglycosides (89.9% vs. 41%), fluoroquinolones (86.7% vs. 29.3), and sulfamethoxazole/trimethoprim (88.1% vs. 26.8%). Among carbapenemase-producers, the most active drug was colistin (97.8%). Among KPC-, IMI- and OXA-48 producers, the most ac-

Table 2
Antibiotic resistance phenotypes in ESCPM species isolates according to EUCAST v. 13.1 breakpoints.

Phenotype	Overall % (n)	*Enterobacter cloacae* complex[1] % (n)	*Klebsiella aerogenes* % (n)	*Enterobacter* non-*cloacae* complex[2] % (n)	*Serratia marcescens* % (n)	*Citrobacter freundii* complex[3] % (n)	*Providencia* species % (n)	*Morganella morganii* % (n)
3GC susceptible[4]	70.2 (4740/6754)	64.6 (1955/3028)	55.5 (497/895)	66.9 (109/163)	88.5 (1356/1532)	72.8 (294/404)	46.2 (79/171)	80.2 (450/561)
3GC resistant[4]	15.7 (1059/6754)	15.2 (459/3028)	29.8 (267/895)	15.3 (25/163)	8.1 (124/1532)	20.5 (83/404)	9.9 (17/171)	15 (84/561)
3GC + 4GC resistant[5]	4.6 (312/6754)	7.4 (225/3028)	3.5 (31/895)	5.5 (9/163)	1.8 (28/1532)	2.7 (11/404)	1.2 (2/171)	1.1 (6/561)
Carbapenems resistant	9.5 (643/6754)	12.8 (389/3028)	11.2 (100/895)	12.3 (20/163)	1.6 (24/1532)	4 (16/404)	42.7 (73/171)	3.7 (21/561)
ESBL-producer	6.4 (374/5832)	11.4 (297/2618)	3.1 (24/779)	4.4 (7/159)	1.9 (25/1296)	3.4 (12/349)	2.9 (4/140)	1 (5/491)
AmpC overproducers	15.8 (641/4056)	17.1 (318/1859)	32.1 (176/549)	17.7 (20/113)	4.8 (43/901)	16.5 (38/230)	13.3 (8/60)	11.1 (38/344)
Carbapenemase-producer	3.1 (205/6713)	3.5 (104/3009)	2.3 (21/896)	1.3 (2/154)	1.1 (16/1521)	2.5 (10/402)	29.4 (50/170)	0.4 (2/561)
KPC-producer	1.5 (3/205)	1 (1/104)	–	50 (1/2)	–	10 (1/10)	–	–
VIM-producer	22.9 (47/205)	39.4 (41/104)	–	–	25 (4/16)	10 (1/10)	2 (1/50)	–
NDM-producer	2.5 (5/205)	3.8 (4/104)	4.8 (1/21)	–	–	–	–	–
IMI-producer	0.5 (1/205)	1 (1/104)	–	–	–	–	–	–
OXA-48-producer	16 (33/205)	11.5 (12/104)	33.3 (7/21)	–	18.8 (3/16)	60 (6/10)	6 (3/50)	100 (2/2)
KPC+VIM-producer	0.5 (1/205)	1 (1/104)	–	–	–	–	–	–
KPC+NDM-producer	0.5 (1/205)	–	–	–	–	10 (1/10)	–	–
Not characterised	55.6 (114/205)	42.3 (44/104)	61.9 (13/21)	50 (1/2)	56.2 (9/16)	10 (1/10)	92 (46/50)	–

Abbreviations: 3GC, third-generation cephalosporins; 4GC, fourth-generation cephalosporins.
[1] *Enterobacter* cloacae complex corresponds to *E. cloacae, E. asburiae, E. hormaechei, E. kobei, E. ludwigii, E. mori*, and *E. Nimipressuralis*.
[2] *Enterobacter* non-cloacae complex corresponds to *Enterobacter* species not included in the *Enterobacter* cloacae complex.
[3] *Citrobacter freundii* complex corresponds to *C. freundii, C. braakii, C. youngae, C. portucalentis, C.gillenii, C. murliniae, C. sedlakii,* and *C. wekmenii*.
[4] Cefepime-and-carbapenem-susceptible.
[5] Carbapenem-susceptible.

Table 3
Antibiotic susceptibility of resistance phenotypes in ESCPM species isolates according to EUCAST v. 13.1.

Phenotype	Antimicrobial susceptibility% (n)								
	FEP	PTZ	C/T	CZA	CARB	CL[1]	AM	FQ	SMX/TMP
3GC susceptible[2]	–	98.1 (4135/4215)	99.6 (508/510)	100 (554/554)	–	93.4 (653/699)	98 (4419/4508)	95.9 (4540/4734)	93.7 (4095/4370)
3GC resistant[2]	–	29.8 (272/914)	67.6 (115/170)	98.2 (222/226)	–	90.3 (187/207)	89.6 (859/959)	87.1 (907/1041)	84.9 (830/978)
3GC + 4GC resistant[3]	–	31.7 (78/246)	66 (35/53)	95.7 (67/70)	–	90.7 (49/54)	44.3 (110/248)	32.9 (102/310)	36.4 (100/275)
Carbapenems resistant	48.8 (300/615)	10.4 (65/623)	15.4 (21/136)	66 (138/209)	–	85.8 (176/205)	65.8 (414/629)	53.8 (343/637)	53.2 (313/588)
Non-carbapenemase-producer	52.9 (227/429)	14.7 (64/437)	25.7 (18/70)	82.8 (106/128)	–	92.9 (92/99)	91.1 (401/440)	74.8 (302/404)	67.3 (280/416)
ESBL-producer	20.9 (71/340)	32.2 (97/301)	58.3 (28/48)	94.3 (83/88)	99.2 (368/371)	93 (53/57)	41 (126/307)	29.3 (109/372)	26.8 (94/351)
AmpC overproducer	84.4 (521/617)	22.1 (118/534)	45.5 (25/55)	98.1 (105/107)	99.2 (636/641)	93.8 (76/81)	89.9 (490/545)	86.7 (556/641)	88.1 (534/606)
Carbapenemase-producer	20.6 (40/194)	1 (2/198)	6.7 (7/105)	33 (37/112)	27.8 (57/205)	97.8 (88/90)	36.6 (74/202)	25 (51/204)	22.4 (41/183)
KPC-/IMI-/OXA-48-producer	44.4 (12/27)	- (0/35)	41.7 (5/12)	100 (22/22)	70.3 (26/37)	93.3 (14/15)	73 (27/37)	37.8 (14/37)	51.7 (15/29)
MBL-producer	34 (18/53)	- (0/52)	- (0/19)	- (0/54)	7.4 (4/54)	96.2 (25/26)	38.5 (20/52)	35.2 (19/54)	22.5 (11/49)

Abbreviations: FEP, cefepime; PTZ, piperacillin/tazobactam; C/T, ceftolozane/tazobactam; CZA, ceftazidime/avibactam; CARB, at least one carbapenem among ertapenem, imipenem and meropenem; CL, colistin; AM, at least one aminoglycoside among gentamycin and amikacin; FQ, either ciprofloxacin or both ciprofloxacin and levofloxacin; SMX/TMP, sulfamethoxazole/trimethoprim; 3GC, third-generation cephalosporins; 4GC, fourth-generation cephalosporins; MBL, metallo-β-lactamases.
[1] Excluding colistin intrinsically resistant species.
[2] Cefepime-and-carbapenem-susceptible.
[3] Carbapenem-susceptible.

tive drugs were ceftazidime/avibactam (100%) and colistin (93.3%), while colistin displayed the highest activity against MBL-producers (96.2%).

ESCPM isolates exhibiting 3GC susceptibility phenotype, 3GC and combined 3GC + 4GC resistance phenotypes were detected mostly from patients admitted in medical wards (37.3%, 35.2% and 42.3%, respectively), whereas carbapenems resistance phenotype was more frequent in critically ill patients (39.2%) (Table S5). The same proportion was maintained in the distribution of resistance mechanisms, as ESBL-producers and AmpC overproducers were more frequently detected in medical ward patients (42.3% and 37.9%, respectively) while carbapenemase-producers were more frequently detected in ICU patients (47.3%). When comparing countries, the highest rates of carbapenems resistance phenotypes were observed in Romania, Switzerland, and Greece (39.2%, 19.9%, and 18.2% respectively) (Figure S2). The highest rates of 3GC resistance phenotype were observed in Denmark, Italy and The Netherlands (23.4%, 22.7%, and 21.8% respectively), whereas those of combined 3GC + 4GC resistance phenotype were mostly detected in Poland, Croatia, and France (9.9%, 9.9%, and 7.9% respectively). Of note, the participating centres from Germany, Denmark, and the Netherlands did not test cefepime in their clinical routine due to internal protocols.

4. Discussion

This study offers a contemporary insight into the diagnostic approach and epidemiology of ESCPM species in Europe during the SARS-CoV-2 pandemic. Its findings may provide support for the development of antimicrobial and diagnostic stewardship strategies, as well as the optimization of current antibiotic treatments. Notable observations that can serve as a foundation for future comparative analyses include: 1) A snapshot of microbiological diagnostics for ESCPM species in Europe, with MALDI-TOF coupled to mass spectrometry being the prevalent method for identification. Limited routine testing for susceptibility to recently approved drugs like ceftazidime/avibactam and ceftolozane/tazobactam, AmpC overproduction characterization, and carbapenemase type identification in some centres. Fosfomycin susceptibility was predominantly determined using broth microdilution although there are no defined recommendations on how to test and report susceptibility to this antibiotic for this group of bacteria. 2) *E. cloacae* complex and *S. marcescens* emerged as the most frequently detected species, a trend also observed among COVID-19 patients. Given the larger bed capacity the overall burden of ESCPM species was most pronounced among medical ward patients. 3) Notably, we identified relevant antimicrobial susceptibility findings, including data on susceptibility to new β-lactam/β-lactamase inhibitor combinations but also to piperacillin/tazobactam and fluoroquinolones.

We observed substantial rates of 3GC and carbapenems resistance phenotypes, while the combined 3GC + 4GC resistance phenotype was less frequent. Although not characterised in all centres, AmpC overproduction emerged as the most frequently detected resistance mechanism, and strains with this characteristic displayed high susceptibility to carbapenems, ceftazidime/avibactam, and colistin. Carbapenemase types contributing to carbapenems resistance were provided in low number, with VIM and OXA-48 enzymes being the most frequently identified. *E. cloacae* complex and *Providencia* species exhibited the most resistant cumulative antimicrobial susceptibility profiles, while *K. aerogenes* showed the highest rate of both 3GC resistant phenotype and AmpC overproduction. ESCPM isolates exhibiting 3GC susceptibility phenotype displayed high susceptibility to piperacillin/tazobactam, aminoglycosides, and fluoroquinolones. In contrast, those displaying 3GC, combined 3GC + 4GC resistance phenotypes and ESBL production exhibited high susceptibility to ceftazidime/avibactam and colistin, besides carbapenems. Colistin was the most active agent against ESCPM isolates (except those intrinsically resistant such as *Serratia, Providencia* and *Morganella*) both displaying carbapenems resistance phenotype and carbapenemase production, with these strains being predominantly isolated in ICU patients.

Infections caused by *Enterobacterales* with the potential for high AmpC β-lactamase expression pose significant challenges for diagnostic and antimicrobial stewardship efforts. Our survey revealed delays in implementing susceptibility tests for recently introduced antibiotics in clinical practice probably also due to local antimicrobial stewardship programs and guidelines. A probable sub-optimal cost-effectiveness in adapting to ESCPM epidemiology the use of workflows including lateral flow immunochromatographic assays, which may be more suitable for the epidemiology of *K. pneumoniae* and *E. coli* [25] was also observed. Furthermore, the adoption of time-consuming susceptibility tests for agents like fosfomycin or β-lactamase characterization tests, specifically AmpC overproduction, appeared to be undervalued. This practice resulted in incomplete and sometimes inaccurate information [26], limiting treatment options [27], emphasizing the need for guidelines in this field. To address this, it is imperative to promptly incorporate susceptibility testing for cefiderocol [28,29] and formulations containing aztreonam and novel β-lactamase inhibitors, especially for MBL producers [30,31], into laboratory workflows. This proactive approach aligns with therapeutic recommendations [15] and facilitates the monitoring of emerging resistance trends.

EUCAST expert rules recommend microbiologists to either discourage the use of 3GC (alone or in combination with aminoglycosides) in their reports or suppress susceptibility testing results for ESCPM species susceptible to cefotaxime, ceftriaxone, or ceftazidime. This is due to the high risk of selecting AmpC derepressed cephalosporin-resistant mutants during therapy. The risk varies, classified as “high” for *Enterobacter* species, *K. aerogenes*, and *C. freundii* complex, “low” or “infrequent” for *M. morganii, Serratia*, and *Providencia* species, and “absent” when cefepime is used [32]. It’s worth noting that broad-spectrum antibiotics, including carbapenems and cefepime, recommended for ESCPM infections, have a substantial ecological impact [8] and potential toxicity [33]. Selecting ESCPM species may be a multifactorial process influenced by antibiotic concentration at the infection site and the involved species. As a result, in case of good diffusion of the antibiotic on the infected site, *M. morganii, Serratia*, and *Providencia* species might be considered candidates for definitive therapy with 3GC, which could significantly impact antibiotic consumption [8]. This has been corroborated for *S. marcescens* in a recent publication [9] but is still a topic of wide debate given the increased risk of treatment failure compared with cefepime/carbapenem [22]. Our study revealed that approximately 40% of strains exhibiting 3GC susceptibility phenotype belonged to these “low risk” species. Furthermore, *E. cloacae* complex, *K. aerogenes*, and *C. freundii* complex displayed low susceptibility to cefotaxime, ceftazidime, ceftolozane/tazobactam, and piperacillin/tazobactam. This may be attributed to various resistance mechanisms, including decreased outer-membrane permeability and concurrent β-lactamase synthesis [34], adding complexity to the empirical selection of these antibiotics against these species. Our study also brought attention to *Providencia* species and its significant burden of antimicrobial resistance. It is worth noting that a substantial portion of these strains might be linked to an uncharacterised outbreak of carbapenemase-producing strains in one of the participating centres.

Given the substantial rates of 3GC and carbapenems resistance phenotypes observed in our study, some considerations must be also made about cefepime and new approved β-lactam/β-lactamase inhibitor combinations as key components of the carbapenem-sparing strategy. Cefepime exhibited potent activity (>90%) against most ESCPM species, except for the *E. cloacae* complex (MIC_{50}–MIC_{90} 1–8 mg/L, EUCAST 82.3%; CLSI 88%), drawing attention to its use against these species, especially when susceptibility is dose dependent (EUCAST clinical breakpoint: susceptible ≤1 mg/L; resistant >4 mg/L) [10]. Ceftazidime/avibactam demonstrated broader activity against ESCPM species compared to ceftolozane/tazobactam, in particular against both ESBL-producers and AmpC overproducers. This finding is noteworthy, as a recent meta-analysis have suggested its effectiveness in ESBL-producing *Enterobacterales* infections and less so in those caused by AmpC producers as compared to carbapenem [35]. Also, our study revealed that the in vitro activity of ceftazidime/avibactam was significantly reduced in isolates displaying both carbapenems resistance phenotype and carbapenemase production. Although its use as monotherapy is discouraged by EUCAST [36], colistin was the most active drug against these strains except for naturally resistant bacterial species, i.e. *Serratia* species, *Morganella morganii* and *Providencia* species. This observation was affected by the prevalence of MBL-producing strains, particularly VIM-type. Other resistance mechanisms, such as $N^{346}Y$ substitutions and deletions in AmpC β-lactamases, were also reported to play a role, leading to resistance to 4GC and reduced susceptibility to cefiderocol, especially following cefepime exposure [37,38]. Additionally, production of minor

carbapenemases, like GIM-1, should not be overlooked [39]. GIM-1 is a MBL identified in Germany in *S. marcescens* [40], *E. cloacae* [41], and *C. freundii* complex [42]. Like other MBLs, it is not inhibited by avibactam [43] and may not be readily characterised by common diagnostic workflows targeting the main carbapenemases.

The present study successfully gathered data from a large multicentre surveillance study, addressing critical gaps in European epidemiological knowledge about these species.

However, some limitations should be acknowledged. Firstly, the study did not assess the incidence of ESCPM species from BCs. This limitation was due to the varied protocols for requesting and performing BCs in different centres, making it challenging to calculate and interpret incidence values. Secondly, susceptibility testing and species identification reported were performed by various methods and this could have affected the integrity of the data since the accuracy of the methods may vary widely [44,45]. Thirdly, the overrepresentation of isolates from France and Spain might skew the regional contribution, as well as the fact that some countries are represented by only one hospital, which could create a bias in case of a local breakthrough. Fourthly, the study faced challenges in reinterpreting MIC values according to both EUCAST and CLSI breakpoints given the automated systems provided results within a limited range (e.g. MIC value >8 mg/L and breakpoint for resistant >32 mg/L) and discrepancies in guidelines for disk antibiotic concentration, particularly for piperacillin/tazobactam, ceftazidime, cefotaxime, and ceftazidime/avibactam. Finally, the reduced number of isolates restricts the generalizability of results, particularly concerning *Providencia* species and its contribution to antimicrobial resistance.

In summary, our study revealed that only a subset of European centres routinely conducted susceptibility testing for new antibiotics, characterised the AmpC overproduction resistance mechanism, and provided identification of carbapenemase enzymes in ESCPM species. We observed a notable burden of *E. cloacae* complex positive BC episodes and antimicrobial resistance. *Providencia* species also exhibited a significant cumulative antimicrobial resistance profile, warranting ongoing and vigilant monitoring. Furthermore, our findings highlighted substantial rates of 3GC and carbapenems resistance phenotypes. Although probably underestimated, AmpC overproduction was the most detected resistance mechanism, with strains featuring this characteristic showing high susceptibility to carbapenems, ceftazidime/avibactam, and colistin. Among carbapenemases, VIM and OXA-48 enzymes were the most frequently identified in ESCPM species. Colistin emerged as the most active drug against ESCPM species (except those intrinsically resistant) displaying both carbapenem resistance phenotype and carbapenemase production. Future European studies should benefit from a centralised characterization of the isolates in order to reduce variability related to the different methods used for species identification and antimicrobial susceptibility testing. It will be then required to address crucial clinical questions, including the impact of rapid diagnostics for ESCPM species on clinical outcomes, the establishment of acceptable risk thresholds for using 3GC against multi-susceptible ESCPM species infections, and the role of carbapenem-sparing antibiotic therapy, especially with new β-lactam/β-lactamase inhibitor combinations.

Declarations

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2024.107115.

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