Ongoing spread of colistin-resistant *Klebsiella pneumoniae* in different wards of an acute general hospital, Italy, June to December 2011

C Mammina (caterina.mammina@unipa.it)[1], C Bonura[1], F Di Bernardo[2], A Aleo[1], T Fasciana[1], C Sodano[2], M A Saporito[2], M S Verde[2], R Tetamo[3], D M Palma[3]

1. Department of Sciences for Health Promotion G D'Alessandro, University, Palermo, Italy
2. Laboratory of Clinical Microbiology, ARNAS General Hospital Civico, di Cristina e Benfratelli, Palermo, Italy
3. II Intensive Care Unit, ARNAS General Hospital Civico, di Cristina e Benfratelli, Palermo, Italy

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We describe polyclonal spread of colistin-resistant *Klebsiella pneumoniae* in an acute general hospital in Italy. Between June and December 2011, 58 colistin-resistant *K. pneumoniae* isolates were recovered from 28 patients admitted to different wards, but mainly in the intensive care units. All isolates were tested for drug susceptibility and the presence of beta-lactamase (*bla*) genes. Clonality was investigated by repetitive extragenic palindromic (rep)-PCR and multilocus sequence typing (MLST). Fifty-two isolates had minimum inhibitory concentrations (MICs) for colistin of 6–128 mg/L, carried bla_{KPC3} and were attributed to sequence type ST258. The remaining six isolates were susceptible to carbapenems, exhibited MICs for colistin of 3–32 mg/L, and belonged to two different types, ST15 and ST273. Rep-PCR included all isolates in three clusters, one containing all ST258 KPC-3-producing isolates and two containing ST15 and ST273 isolates. Cross-transmission containment measures and intensification of staff and environmental hygiene could not stop the outbreak. Selective pressure and horizontal transmission probably contributed to emergence and spread of three different strains of colistin-resistant *K. pneumoniae* in the hospital. Strict implementation of the above measures and a wider awareness of the antimicrobial resistance threat are crucial to preserve the last therapeutic options of the multidrug-resistant Gram-negative infections.

Introduction

Infections caused by Gram-negative pathogens resistant to carbapenems are emerging as a consequence of the intensive use of these compounds [1]. *Klebsiella pneumoniae* carbapenemase (KPC)-producing organisms in particular are of great concern from a public health and clinical point of view, because these infections have a high crude mortality rate and the therapeutic options are limited [2,3]. Most of them are susceptible only to gentamicin, polymyxins and tigecycline [2,3].

Polimyxins are considered as the last resort for treatment of infections with carbapenem-resistant Gram-negative bacteria. However, resistance to these compounds has begun to emerge, although infrequently [4-8]. Selective pressure by the increased use of colistin and clonal expansion through horizontal transmission have generated clusters of cases infected with multiresistant *K. pneumoniae* strains that have been generally attributed to the international epidemic clone ST258 [4,7].

In Italy, KPC-producing *K. pneumoniae* strains are endemic [9]. After the first report in 2008 [10], KPC-producing *K. pneumoniae* have widely spread, and several hospital outbreaks have been described [7,11-14]. Recently the European Antimicrobial Resistance Surveillance Network (EARS-Net)'s surveillance system for Italy has shown that the prevalence of carbapenem resistance in *K. pneumoniae* has risen from 1–2% in the years 2006–09 to 15% in 2010 [15]. An outbreak caused by colistin-resistant isolates of KPC-producing *K. pneumoniae* belonging to sequence type ST258 has also been described [7].

In 2008, the II intensive care unit (ICU) of the ARNAS general hospital Civico, di Cristina e Benfratelli in Palermo, Italy, was affected by the first outbreak of KPC3-*K. pneumoniae* ST258 occurring in Sicily [14]. Since then, after a brief transient period during which the outbreak strain was not detected, colonisations or infections with KPC3-*K. pneumoniae* have become endemic in the hospital.

Here we describe the polyclonal spread of colistin-resistant *K. pneumoniae* on different wards of the same hospital in the period from June to December 2011.

Materials and methods

Setting

The ARNAS general hospital Civico, Di Cristina e Benfratelli in Palermo is the largest acute general hospital in Sicily. It had during the study period (June to

December 2011) a capacity of 901 beds in total, including 24 beds in two general intensive care units (ICUs), identified as I and II ICU. Each unit has dedicated nursing and medical staff. At the time of the outbreak, the infection control policy in the ICUs did not include routine surveillance cultures or screening of high risk patients on admission. Special attention was given to hand hygiene measures, with an alcoholic hand rub solution placed in the proximity of every ICU bed or provided as a personal pocket dispenser. Furthermore, the hospital did not have a policy of restricting the use of antibiotic drugs or clinical practice guidelines for infections with multidrug-resistant pathogens. The hospital microbiology laboratory regularly provided susceptibility results to first and second line antibiotics, but a structured system for the surveillance of antimicrobial resistance had not been implemented. However, a hospital formulary and an antibiotic policy committee were present. Antibiotic consumption was monitored through data from the hospital's pharmacy, but the defined daily dosage (DDD) was not measured.

Microbiology and molecular epidemiology

All colistin-resistant *K. pneumoniae* isolates, irrespective of their source patient and clinical sample, were subjected to further phenotypic and molecular analysis. Data about time and ward of isolation, clinical sample and antibacterial drug susceptibility pattern were evaluated using the records of the microbiology laboratory.

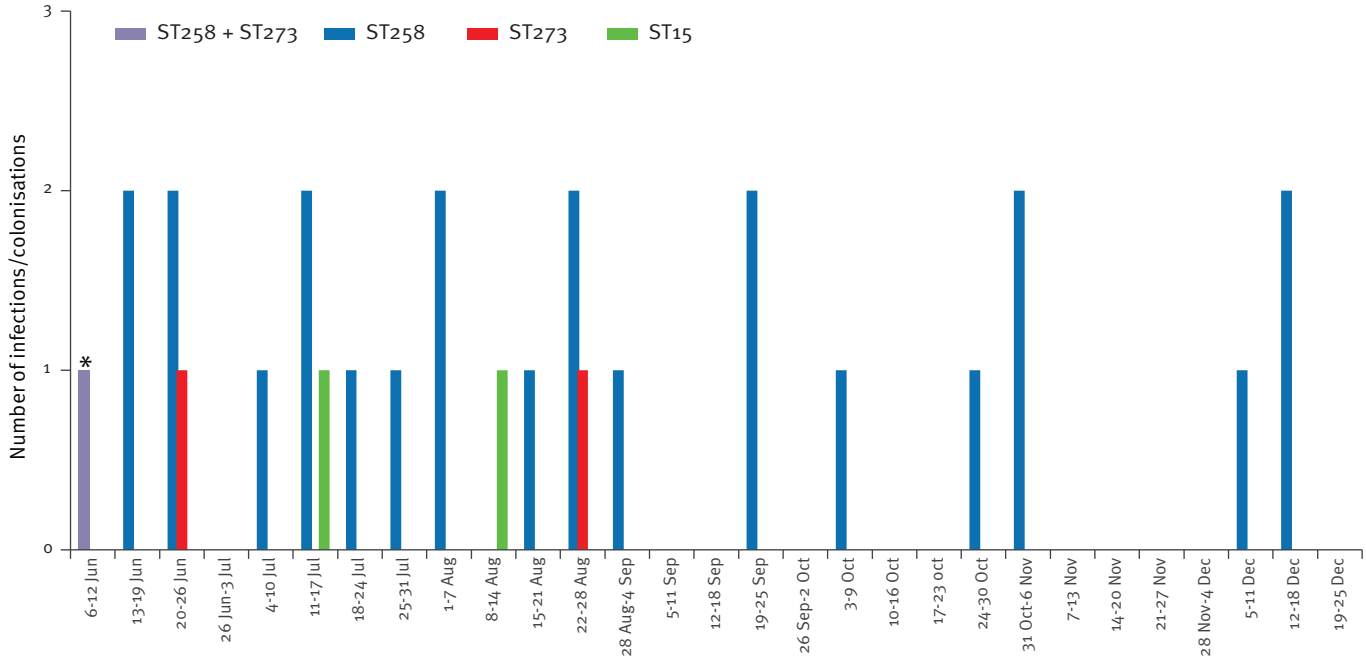
Bacterial identification and antibiotic susceptibility testing were initially performed at the Laboratory of Microbiology of the ARNAS general hospital Civico, di Cristina e Benfratelli using the VITEK-2 automated system (bio-Mérieux, Marcy l'Etoile, France). Minimum inhibitory concentrations (MICs) of imipenem (IMP), meropenem (MEM) and colistin (CT) were also determined by using Etest (AB Biodisk, Solna, Sweden), following the guidelines of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [16]. *Escherichia coli* ATCC 25922 was used as quality control strain. Susceptibility and resistance categories were assigned according to the EUCAST breakpoints [16]. Phenotypic screening for the presence of carbapenemases or overexpression of AmpC in combination with porin loss was performed by a commercial synergy test (Rosco Diagnostica, Taastrup, Denmark).

The isolates were subjected to polymerase chain reaction (PCR) to screen for the presence of bla_{KPC}, bla_{SHV}, bla_{TEM}, bla_{CTX-M}, bla_{VIM} and bla_{IMP}, as described previously [17]. DNA sequencing was performed on both strands of the PCR amplification products. Results were compared and aligned with reference sequences using the online BLAST database and CLUSTAL W software.

Clonality of the *K. pneumoniae* isolates was investigated by molecular typing, using the repetitive extragenic palindromic (rep)-PCR methodology. Rep-PCR was performed using the DiversiLab system (bioMérieux,

Figure 1

Infections/colonisations with colistin-resistant *Klebsiella pneumoniae* strains, ARNAS general hospital Civico, di Cristina e Benfratelli, Palermo, June–December 2011(n=28)



All cases are represented by week, based on the day of first isolation of the colistin-resistant isolate.
The asterisk designates the index patient from whom two isolates belonging to different sequence types (ST258 and ST273) were isolated.

TABLE

Characteristics of colistin-resistant *Klebsiella pneumoniae* isolates, ARNAS general hospital Civico, Di Cristina e Benfratelli, Palermo, June–December 2011 (n=58)

Case	Isolate(s)	Time of isolation	Clinical sample(s)	Ward	Minimum inhibitory concentration[a] (mg/L)					*bla* genes	ST
					IPM	MEM	TG	CT	GM		
1	1	8 June	UR	I ICU	≥16	≥16	1	128	4	KPC-3, SHV-11, TEM-1, OXA-9	258
	5	20 June	BA		≤1	≤0.25	2	3	16	SHV-12, TEM-1	273
2	2	17 June	CVC	U	≥16	≥16	0.5	24	4	KPC-3, SHV-11, TEM-1, OXA-9	258
3	3, 7	18, 20 June	BS, BA	I ICU	1	≤0.25	≤0.5	3	≥16	SHV-12, TEM-1	273
4	4, 23, 38, 42, 50	20 June, 17 August, 21 September, 5 October, 13 November	BA (4), UR (23, 38, 42, 50)	I ICU	≥16	≥16	1–2	12–32	‹1–4	KPC-3, SHV-11, TEM-1, OXA-9	258
5	6	20 June	BA	I ICU	≥16	≥16	1	16	2	KPC-3, SHV-11, TEM-1, OXA-9	258
6	8, 13	14, 22 July	BA, BS	HA	≥16	≥16	1–2	24	4	KPC-3, SHV-11, TEM-1, OXA-9	258
7	9	12 July	UR	II ICU	≤1	≤0.25	2	4	≥16	SHV-28, TEM-1, CTX-M-15	15
8	10	12 July	BA	CS	≥16	8	2	128	4	KPC-3, SHV-11, TEM-1, OXA-9	258
9	11, 15, 22	8, 20 July, 16 August	UR	I ICU	≥16	≥16	1–2	16–32	2–4	KPC-3, SHV-11, TEM-1, OXA-9	258
10	12, 19	26 July, 16 August	BS	NS	≥16	≥16	1	32,48	4–8	KPC-3, SHV-11, TEM-1, OXA-9	258
11	14	21 July	BS	I ICU	≥16	≥16	1	24	4	KPC-3, SHV-11, TEM-1, OXA-9	258
12	16, 29, 31, 35, 39	4, 29 August, 5, 20 September	BA (16, 31, 35, 39), UR (29)	I ICU	8–≥16	≥16	≤0.5–1	16–24	4–≥16	KPC-3, SHV-11, TEM-1, OXA-9	258
13	18	3 August	WS	PS	8	≥16	2	16	4	KPC-3, SHV-11, TEM-1, OXA-9	258
14	20, 24	16 August	BA, CVC	II ICU	≥16	≥16	1	24	4	KPC-3, SHV-11, TEM-1, OXA-9	258
15	21	10 August	BA	RICU	≤1	≤0.25	2	12	≥16	SHV-28, TEM-1, CTX-M-15	15
16	25	22 August	BA	I ICU	≤1	≤0.25	2	32	≥16	SHV-12, TEM-1	273
17	26, 27	24, 25 August	BA,BS	II ICU	≥16	≥16	2	12,3	4–≥16	KPC-3, SHV-11, TEM-1, OXA-9	258
18	28, 33	28 August, 10 September	BS, SW	I ICU	≥16	4–≥16	≥16	32,8	‹1–4	KPC-3, SHV-11, TEM-1, OXA-9	258
19	32, 37, 40	3, 21 September, 4 October	UR	I ICU	≥16	≥16	1–2	12–32	‹1–4	KPC-3, SHV-11, TEM-1, OXA-9	258
20	34	20 September	BA	II ICU	4	8	1	24	2	KPC-3, SHV-11, TEM-1, OXA-9	258
21	36	21 September	BS	NS	≥16	≥16	1	32	4	KPC-3, SHV-11, TEM-1, OXA-9	258
22	41, 43	5, 14 October	UR (41), BS (43)	I ICU	≥16	≥16	2	16,32	‹1–4	KPC-3, SHV-11, TEM-1, OXA-9	258
23	44	24 October	UR	I ICU	≥16	≥16	2	6	4	KPC-3, SHV-11, TEM-1, OXA-9	258
24	45, 47, 48, 49, 51, 52, 54, 57, 58	31 October, 7, 14, 22 November, 2, 12, 22, 23 December	CSF (45, 47, 48, 51, 52, 54, 57, 58), CVC (49)	I ICU	4–≥16	4–≥16	1–2	24–32	2–4	KPC-3, SHV-11, TEM-1, OXA-9	258
25	46	31 October	CSF	I ICU	≥16	≥16	2	32	1–2	KPC-3, SHV-11, TEM-1, OXA-9	258
26	53, 56	5, 23 December	BA	I ICU	≥16	≥16	1	32	4	KPC-3, SHV-11, TEM-1, OXA-9	258
27	55	12 December	BA	I ICU	≥16	≥16	1	16	4	KPC-3, SHV-11, TEM-1, OXA-9	258
28	59, 60	17, 21 December	UR	II ICU	≥16	≥16	1	16	4	KPC-3, SHV-11, TEM-1, OXA-9	258

BA: bronchial aspirate; BS: bloodstream; CS: cardiac surgery; CSF: cerebrospinal fluid; CT: colistin; CVC: central venous catheter; GM: gentamicin; HA: haematology; ICU: intensive care unit; IPM: imipenem; KPC: Klebsiella pneumoniae carbapenemase; MEM: meropenem; NS: neurosurgery; PS: plastic surgery; RICU: respiratory intensive care unit; TG: tigecycline; U: urology; UR: urine; WS: wound swab.

[a] One figure is indicated when there were no differences between the strains isolated from a given patient. When two different values were obtained, both figures are indicated here. Results for more than two isolates are shown as a range.

Marcy l'Étoile, France). The model 2100 bioanalyzer and LabChip reagents (Agilent Technologies Inc., Palo Alto, CA) were used for DNA separation and sizing. DNA fragment patterns were then analysed by using the modified Kullback-Leibler coefficient pairwise pattern matching and the unweighted pair group method with arithmetic mean (UPGMA) clustering algorithm [18]. In addition, multilocus sequence typing (MLST) was performed and sequence types were determined using the *K. pneumoniae* MLST database [19].

Results

Between June and December 2011, 58 colistin-resistant *K. pneumoniae* isolates were recovered from 28 patients. The index case was a patient staying in the I ICU, from whom a colistin-resistant *K. pneumoniae* isolate was identified on 8 June 2011. The epidemic curve of infections/colonisations with colistin-resistant *K. pneumoniae* strains in the hospital is shown in Figure 1.

The main characteristics of the isolates are summarised in the Table. Because the index case proved to be infected by two different clones of colistin-resistant *K. pneumoniae*, all isolates from subsequent cases were submitted to phenotypic and genetic analysis.

All isolates, except for six (isolates number 3, 5, 7, 9, 21 and 25 from five different patients) were resistant to all beta-lactams and beta-lactam/beta-lactamase inhibitor combinations (ampicillin, ampicillin/sulbactam, amoxicillin/clavulanic acid, third generation cephalosporins, piperacillin, piperacillin/tazobactam, ticarcillin, ticarcillin/clavulanic acid, aztreonam, imipenem, meropenem), fluoroquinolones (ciprofloxacin, levofloxacin), co-trimoxazole and aminoglycosides (amikacin, netilmicin, tobramycin). Only 10 of the 52 isolates were found to be susceptible to gentamicin. All 52 isolates were resistant to colistin (Table), with MICs ranging from 3 mg/L to 128 mg/L.

Phenotypic screening for the presence of carbapenemases or AmpC/porin loss suggested KPC production. PCR analysis and nucleotide sequencing revealed that the 52 isolates carried the bla_{KPC-3} gene as well as an SHV-11 extended-spectrum beta-lactamase (ESBL) enzyme. MLST attributed these isolates with the sequence type ST258 (Table).

The remaining six isolates were resistant to beta-lactams and beta-lactam/beta-lactamase inhibitor combinations, but susceptible to carbapenems (Table). They were also susceptible to cotrimoxazole, and resistant to fluoroquinolones and aminoglycosides, except amikacin. MICs of colistin ranged from 3 mg/L to 32 mg/L. MLST attributed the six isolates to two different STs, ST15 and ST273. PCR analysis and nucleotide sequencing of the amplicons identified bla_{SHV-12} in the four ST273 *K. pneumoniae* isolates, whereas bla_{SHV-28} and $bla_{CTX-M-15}$ were detected in the two ST15 isolates (Table).

Rep-PCR confirmed that the colistin-resistant isolates belonged to three different clusters, one that contained all ST258 KPC-3 producing isolates, and two clusters with unrelated patterns including the ST15 and ST273 isolates (Figure 2).

Control measures

During the period from June to December 2011, colistin-resistant ST258 KPC-3-producing *K. pneumoniae* spread on six different wards of the acute general hospital Civico, di Cristina e Benfratelli, although its epicenter was in the I ICU, where 16 of 28 patients were staying at the time of the isolation of the colistin-resistant strain (Table).

Infection control measures were strengthened on all affected wards following the detection and confirmation of the first colistin-resistant *K. pneumoniae* isolate. In particular, hand hygiene, contact precautions were improved, including dedicated use of patient¬ care equipment and use of disposable gloves and aprons, and environmental cleaning. The ICUs were thoroughly cleaned and respiratory equipment disinfected. Because of insufficient bed capacity, isolation was not feasible in either ICU, but colonised or infected patients were cohorted in the II ICU, and spatially segregated in the I ICU as well as the other wards. Compliance with hand hygiene and environmental cleaning procedures were monitored. Adherence of the personnel was strongly promoted by weekly meetings between healthcare workers and the hospital infection control team.

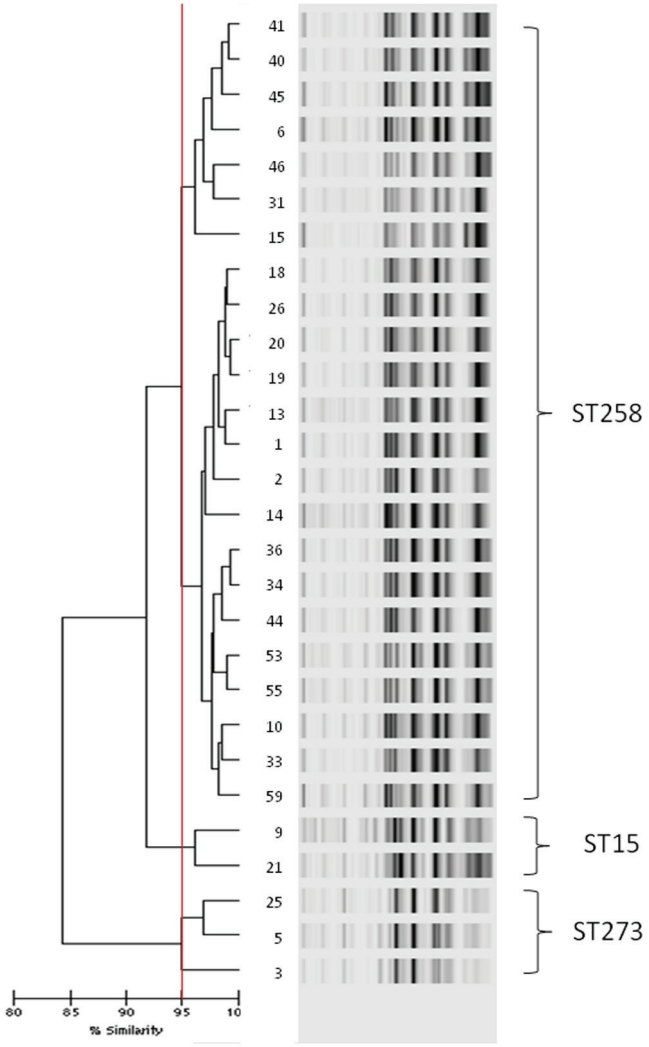
Despite these measures, the outbreak developed further, and additional isolates were detected. The outbreak peaked in July and August 2011, but new cases occurred in the following months. In December 2011, three new cases were still recognised (Table and Figure 1).

Discussion

Emergence of colistin-resistant strains among the expanding population of multi-resistant Gram-negative bacteria is an inevitable consequence of the increasing use of this antimicrobial agent [20]. Mono- or multiclonal outbreaks of colistin-resistant, KPC-producing *K. pneumoniae* have been described in hospitals in many countries, for example Greece, South Korea and the United States [4-6,8]. In Italy, an outbreak involving eight patients was reported in 2010. It occurred in two different hospitals in Catania, the same region in Sicily also affected by the ongoing outbreak described here [7]. Most often, outbreaks due to KPC-producing *K. pneumoniae* have been attributed to the ST258 clone. Also in our case, a KPC-3 producing, ST258 strain was responsible for the largest proportion of infections/colonisations, and cross-transmission between patients was likely to be the key factor of its clonal expansion. We hypothesise that the main obstacles preventing interruption of the transmission chain were the extended length of stay of some critically ill patients in

Figure 2

Rep-PCR analysis of colistin-resistant *Klebsiella pneumoniae* isolates, ARNAS general hospital Civico, di Cristina e Benfratelli, Palermo, June–December 2011 (n=28)



The dendrogram includes 23 representative KPC-3-producing K. pneumoniae isolates from 23 different patients and five carbapenem-susceptible K. pneumoniae isolates from five different patients. Isolates with ≥95% similarity were considered related.

the ICU as long-term reservoirs of multidrug-resistant organisms, as well as the need to move some patients between different wards of the hospital.

A further reason for concern was the detection of other *K. pneumoniae* isolates, belonging to ST15 and ST273, that were resistant to colistin, albeit at lower MICs than the ST258 clone, and susceptible to carbapenems. ST15 isolates with an indistinguishable genetic antibiotic resistance pattern have recently been reported as epidemic in Denmark [21]. Moreover, metallo-beta-lactamase producing *K. pneumoniae* strains of ST273, along with ST147, have recently been exported from southern Europe to Scandinavia [22]. ST273 is a single-locus variant of ST147 that has previously been associated with CTX-M-15-producing isolates from several countries, for example Hungary [23], Italy [24], Spain [25] and Tunisia [26]. An ST147 *K. pneumoniae* producing NDM-1 and CTX-M-15 has been recently reported in a patient returning from India to Canada, suggestive of the likely prominent role of this clone in the intercontinental dissemination of antimicrobial resistance [27]. However, none of our patients carrying these strains (ST15 and ST273) had a history of recent travel to other European countries. Consequently, in our setting colistin resistance is more likely to have emerged through selective pressure and spread through cross-transmission. It is also noteworthy that colistin resistance involved strains belonging to globally successful multi-resistant clonal complexes [28].

Treatment with colistin and its duration have been proven to be major risk factors for the emergence of resistance. They have also been associated with other multi-resistant Gram-negative organisms intrinsically resistant to colistin, such as *Proteus*, *Providencia* and *Morganella* [20]. A further reason for concern is the parallel development of colistin resistance in *Acinetobacter baumannii*, a possible co-infecting or co-colonising organism found mainly in ICU patients and other critically ill patients [29].

With the increasing need to use colistin for the treatment of multidrug-resistant Gram-negative pathogens, it is likely that healthcare-associated emergence and spread of colistin resistance will rise. Implementation of strict infection control measures, antibiotic stewardship and widespread promotion of better awareness of the danger of antimicrobial resistance, are crucial to preserve the last therapeutic options for infections with multidrug-resistant Gram-negative bacteria.

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