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Colistin-resistant isolates of *Klebsiella pneumoniae* emerging in intensive care unit patients: first report of a multiclonal cluster

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***Objectives*: Infections due to multidrug-resistant (MDR) Gram-negative pathogens in the ICU have prompted the use of colistin, an antibiotic forgotten for decades. The aim of this retrospective observational study was to record and present the emergence of colistin-resistant *Klebsiella pneumoniae* (CRKB) in a Greek ICU.**

***Methods*: In a new university tertiary hospital, the first patients admitted in the ICU were already colonized or infected with MDR pathogens, and this led to frequent colistin use as part of empirical or microbiologically documented therapy. Colistin resistance was defined as MIC >4 mg/L by the Etest method. All CRKB isolated in surveillance cultures or clinical specimens in the ICU during the period 2004–5 were recorded along with patients' characteristics.**

***Results*: Eighteen CRKB were isolated from 13 patients over a 16 month period, representing either colonizing or infective isolates. Patients' mean age was 70 years, with a mean APACHE II score at admission of 22. They all had a long hospitalization (median 69 days) and a long administration of colistin (median 27 days). Colistin-resistant isolates were implicated as pathogens in two bacteraemias, a ventilator-associated pneumonia and two soft tissue infections. Repetitive extragenic palindromic PCR identified six distinct clones, and horizontal transmission was also documented.**

***Conclusions*: Selective pressure due to extensive or inadequate colistin use may lead to the emergence of colistin resistance among *K. pneumoniae* isolates, jeopardizing treatment options in the ICU, potentially increasing morbidity and mortality of critically ill patients and necessitating prudent use of colistin.**

Keywords: polymyxins, microbial resistance, antibiotic consumption, selection pressure

Introduction

The emergence of multidrug-resistant (MDR) Gram-negative pathogens has been increasingly described worldwide.[1] The recovery of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* isolates susceptible only to polymyxins from critically ill patients has led to the revival of colistin, an antimicrobial forgotten for decades, which appears as the only treatment choice either empirically or as microbiologically documented therapy.[2]

The appearance of metallo-β-lactamase (MBL)-producing Enterobacteriaceae since 2001 in Greek ICUs and especially of *Klebsiella pneumoniae*[3] has resulted in excessive empirical use of colistin. Herein, a cluster of multiclonal *K. pneumoniae* isolates with acquired resistance to colistin is presented.

Materials and methods

Setting

University general hospital 'ATTIKON' is a new tertiary teaching hospital in Athens, with a potential of 750 beds in total, including a

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general (medical–surgical) 24 bed ICU. The hospital's operation started in the autumn of 2003, with 320 beds currently operating, of which 6 belong to the ICU.

Infection control and antibiotic policies in the ICU include the performance of surveillance cultures (bronchial secretions, stool and urine samples) biweekly. All specimens (except blood and stool) are cultured quantitatively. The diagnosis of ventilator-associated pneumonia (VAP) is supported by bronchoscopy and bronchoalveolar lavage (BAL). All Gram-negative isolates are screened for extended-spectrum β-lactamase (ESBL) activity after applying the double disc approximation test[4] and for MBL production by the EDTA-imipenem disc synergy test.[5]

Infectious diseases consultation is provided on a daily basis and special attention is given to the implementation of proper hand hygiene measures by the use of an alcoholic hand-rub solution, placed on every ICU bed-rail.

The first patients admitted to the ICU in November 2003 were transferred from other hospitals, already harbouring MDR Gram-negative pathogens (*P. aeruginosa*, *A. baumannii*, *K. pneumoniae*) susceptible only to colistin, either as colonizers or infecting microorganisms. This prompted the frequent use of colistin in the ICU, either as empirical therapy guided by the result of the surveillance cultures or as microbiologically documented treatment.

Microbiology

All colistin-resistant *K. pneumoniae* (CRKB) isolates from patients hospitalized in the ICU during 2004 and 2005 were retrospectively recorded. Identification was performed using routine microbiological methodologies and an automated identification system (API ID32GN and ID32E system, bioMérieux, Marcy l'Étoile, France). Colistin MICs were evaluated using the Etest methodology (AB Biodisk, Solna, Sweden).

Resistance to colistin was defined as MIC >4 mg/L, according to the MIC breakpoints of the BSAC for *Acinetobacter* and Enterobacteriaceae.[6]

The ESBL activity, detected by the double disc approximation test, was confirmed as recommended by the CLSI.[6] Isolates with a positive EDTA-imipenem disc synergy test were subsequently evaluated for the presence of a bla_{VIM} gene by PCR amplification.[7]

The clonal profile of the *Klebsiella* isolates was investigated by molecular typing, which was performed using repetitive extragenic palindromic (REP)-PCR methodology.[8]

Patients

The demographic, clinical characteristics and outcome of patients harbouring the colistin-resistant *Klebsiella* isolates were evaluated using patients records.

Consumption of colistin in the ICU (in DDDs/1000 patient-days) between November 2003 and December 2005 was also calculated using the hospital's pharmacy records.

Results

Six months after the first patient's admission to the ICU in November 2003, the first *K. pneumoniae* isolate resistant to colistin was recorded in May 2004. Between May 2004 and August 2005, 18 *K. pneumoniae* isolates resistant to colistin were recovered from 13 patients. Characteristics of patients and isolates are presented in Table 1.

Microbiology—the isolates

Eighty-three percent (15/18) of the *Klebsiella* isolates produced ESBL, 72% (13/18) MBL and 61% (11/18) both. The majority (13/18) represented colonization except for 5 cases of infection where CRKB was implicated as the pathogen in ICU-acquired infections. Colistin MICs ranged between 12 and >1024 mg/L.

Using REP-PCR, 6 distinct clones (A–F) were identified among the 18 isolates (Figure 1). Clones A, B, C and F appeared concomitantly in May 2004 in three patients, as a cluster, after a peak in colistin consumption in the ICU (Figure 1). Different clones were recorded to coexist in patients (Table 1). During the following 6 months (May–November 2004), a horizontal transmission of Clone C to four patients and Clone A to three patients was recorded. Clones D and E appeared many months later (Figure 1) and were horizontally transmitted to two patients (one each). All CRKB clones vanished after the patients who harboured them either died or left the ICU.

Patients

All patients harbouring CRKB had a long ICU stay (median of 69 days) and a significant exposure to colistin (median 27 days) at the time of isolation of the colistin-resistant strain. All three patients who had a short ($\leq$4 days) exposure to colistin represented examples of horizontal transmission of CRKB. Patients had a male to female ratio of 1.6, a mean age of 70 years and a mean APACHE II score at admission of 22, and all were mechanically ventilated during the duration of their ICU stay (Table 1). Nine of the 13 patients were on continuous veno-venous haemofiltration (CVVH), and colistin was administered in dosages from 3 to 9 MU/day according to renal function values. No plasma levels were available.

Five infections due to CRKB were recorded in four patients. Two of them were soft tissue infections (an infected gangrene due to heparin-induced thrombocytopenia and a post-surgical wound infection), a VAP, a central venous catheter-related bacteraemia and a primary bacteraemia. In three of the five cases of infection, there were only tetracyclines left as treatment options, which were used in the form of intravenous doxycycline and tigecycline (Table 1). The primary bacteraemia case failed to respond to treatment with tigecycline and had a fatal outcome. Eleven of the 13 patients eventually died in the ICU.

Discussion

Development of antimicrobial resistance is a phenomenon inevitably related to microbial evolution and antibiotic use.[1] The potential to generate resistance to colistin has been both reported to be slow and low and data on acquired resistance to colistin are limited.[9]

Klebsiella resistant to colistin is rarely described either as a laboratory procedure[10] or in selected reports of epidemics in nurseries several decades ago, when colistin was used as prophylaxis or for decontamination of the digestive tract in neonates.[11,12] At that time, colistin resistance was clearly related to selection pressure from colistin use.

Recently, two probable infections caused by *Klebsiella* resistant to colistin have been cited, isolated in ICU patients, but in these cases, documentation of infections is not fully provided.[13] We present the first cluster of *K. pneumoniae* isolates

Table 1. CRKP isolates: *in vitro* and clinical characteristics

Patient	Age	APACHE II at admission	MV	CVVH	Isolate clone	Source	ESBL	MBL	Colistin DD (mm)	Colistin MIC (mg/L)	Days in ICU at isolation	Days of colistin treatment at isolation	Colonization/ infection	Treatment for the infection	Outcome of infection	Overall outcome
1	71	32	+	+	C	BRS	+	−	12	24	63	0	colonization			good (left ICU)
2	69	38	+	−	B	BRS	−	−	10	24	24	23	colonization			
					B	faeces	+	+	9	32	28	27	colonization			
					C	blood	+	+	10	96	28	27	bacteraemia	intravenous tetracycline	cure	death from other infection
3	75	47	+	+	B	BRS	+	−	10	256	49	29	colonization			
					F	pus	+	+	10	64	53	33	STI	intravenous tetracycline	stable	death
4	84	12	+	+	C	BAL	−	+	13	96	21	4	VAP	imipenem	improvement	death from other infection and GI bleeding
5	63	18	+	+	A	BRS	+	+	15	24	13	18	colonization			death
6	57	5	+	−	C	urine	+	+	13	24	39	17	colonization			death
7	75	20	+	+	A	urine	+	+	10	64	65	45	colonization			death from
					C	pus	−	−	13	64	65	45	STI	imipenem	stable	infection with
					C	blood	+	+	7	128	65	45	bacteraemia	tigecycline	failure	colistin-resistant isolate
8	59	12	+	+	A	faeces	+	+	0	16	32	30	colonization			death
9	67	34	+	+	A	urine	+	+	12	>1024	31	30	colonization			death
10	80	18	+	+	D	faeces	+	+	12	24	24	15	colonization			good (left ICU and hospital)
11	59	11	+	−	E	BRS	−	+	14	12	115	19	colonization			death
12	76	20	+	+	D	BRS	+	−	14	16	65	28	colonization			death
13	76	18	+	−	E	BRS	+	+	0	12	48	0	colonization			death

STI, soft tissue infection; DD, disc diameter; BRS, bronchial secretions; MV, mechanical ventilation; CVVH, continuous veno-venous haemofiltration.

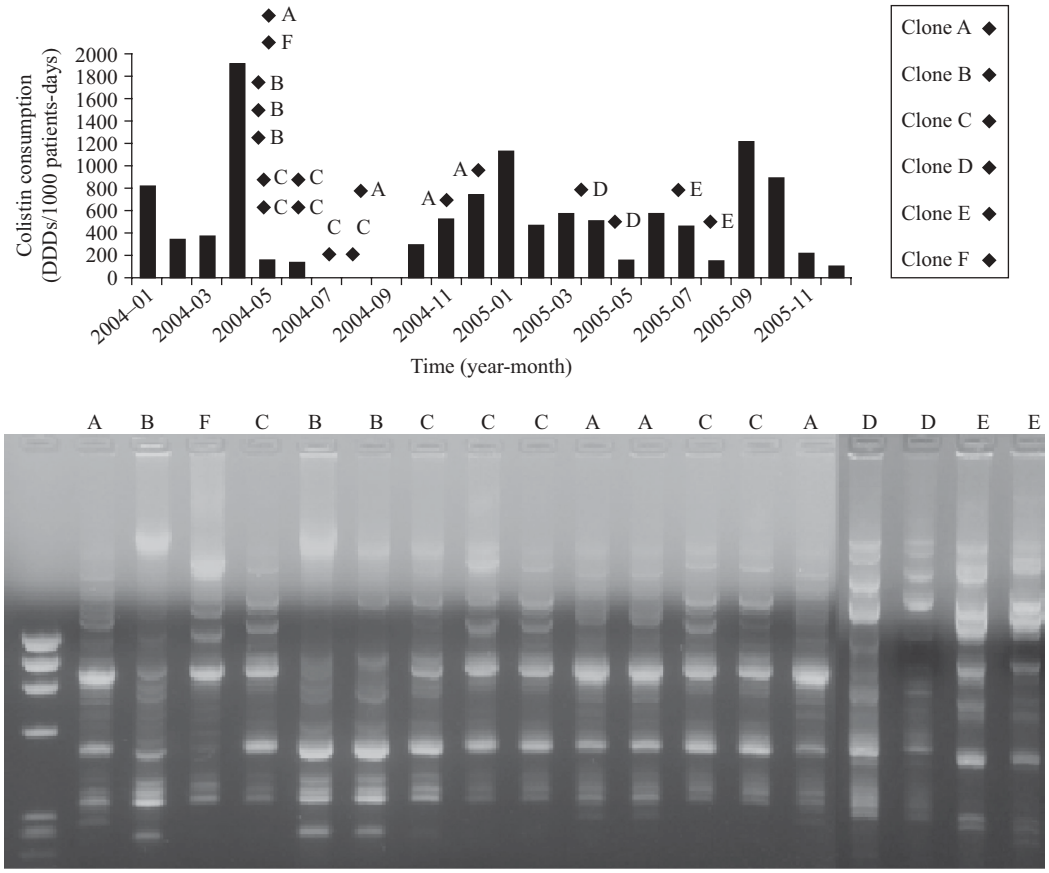

Figure 1. Colistin consumption (DDDs/1000 patient-days) combined with timing of isolation of CRKP isolates and the six distinct clones (A–F) of the 18 CRKP isolates as visualized by REP-PCR. Lane 1, Φx174/*Hae*III.

significantly resistant to colistin. All strains had MICs >8 mg/L (range 12 to >1024 mg/L), clearly resistant by all known breakpoints. The cluster was multiclonal and emerged in critically ill patients, hospitalized in the ICU, already colonized with *K. pneumoniae* strains and who were exposed to prolonged treatment with colistin, a scenario routinely prevalent in Greek ICUs. Horizontal transmission to other concurrently hospitalized patients was also seen, in a form that could be described as a small-scale epidemic, underlining the future threat of resistance to spread and become an endemic phenomenon.

Most importantly, these resistant strains exhibited the potential to cause infections with limited alternative treatment choices. All patients with infections suffered from critical illnesses and had a crude mortality of 100%. Mortality attributed to infection from CRKB was recorded in one patient (25%). In ICU environments where MBL/ESBL-producing microorganisms are increasingly isolated and colistin is the empirical and/or microbiologically documented treatment of choice, the emergence of colistin resistance poses a realistic threat compromising treatment choices and potentially the outcome of critically ill patients.

The hypothesis that acquired resistance to colistin was due to selection pressure from excessive or inadequate colistin use is supported by the concurrent and multiclonal appearance of resistance in patients heavily treated with colistin, previously harbouring colistin-susceptible strains. The level of colistin consumption or plasma levels representing the breakpoint for the emergence of resistance are not easily defined. Documentation of colistin use and parameters such as PK/PD as risk factors for the emergence of resistance against colistin must be the objective of future studies.

Intensification of hand and environmental hygiene measures and the probable non-plasmid-mediated mechanisms of colistin resistance, an issue that also needs further investigation, prevented the uncontrolled spread of colistin-resistant isolates.

Conclusions

Excessive and prolonged or inadequate colistin use in the setting of critically ill patients with multiresistant Gram-negative pathogens may lead to the emergence of colistin resistance concerning not only colonizers but also strains capable of causing serious infections for which there are few or no treatment choices, potentially increasing morbidity and mortality in this patient population. The latter events urge for the development of new antimicrobials against multiresistant Gram-negative pathogens, the prudent use of colistin and the strict implementation of hand hygiene rules.

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Transparency declarations

None to declare.

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