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CASE REPORT

Emergence of OXA-48 and OXA-181 Carbapenemases among *Enterobacteriaceae* in South Africa and Evidence of *In Vivo* Selection of Colistin Resistance as a Consequence of Selective Decontamination of the Gastrointestinal Tract

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This study reports on the emergence of OXA-48-like carbapenemases among isolates of *Enterobacteriaceae* in South Africa. In addition, the emergence during therapy of a colistin-resistant OXA-181-producing *Klebsiella pneumoniae* isolate was documented following selective digestive tract decontamination with oral colistin, which is therefore strongly discouraged.

CASE REPORTS

On 3 August 2011, a 45-year-old male, insulin-dependent diabetic was transferred to an intensive care unit (ICU) at a private hospital in Johannesburg, South Africa, with peripheral vascular disease and sepsis. The patient had previously had a kidney transplant in Egypt, where he had been intermittently hospitalized for 10 months prior to his admission in Johannesburg. Empirical therapy with meropenem (500 mg every 8 h as an extended 4-h intravenous infusion [IVI]) and teicoplanin (6-mg/kg loading dose followed by 6 mg/kg every 12 h IVI) was initiated. A surgical debridement was performed within 48 h of admission, and a culture of an intraoperative tissue specimen from his left groin grew a *Klebsiella pneumoniae* strain that, according to automated susceptibility testing (Vitek 2; bioMérieux, Johannesburg, South Africa), was resistant to most commonly used antibiotics (i.e., aminopenicillins, β-lactam/β-lactamase inhibitors, fluoroquinolones, cephalosporins, and carbapenems). Subsequent disc susceptibility testing, according to the Clinical and Laboratory Standards Institute (CLSI) (1) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints (2), showed that this isolate was susceptible only to amikacin, tigecycline, and colistin. MICs were determined by Etests (AB bioMérieux, Johannesburg, South Africa) on Mueller-Hinton agar at 37°C and interpreted according to CLSI standards (1) except for tigecycline, for which the U.S. Food and Drug Administration recommendations (≤2 μg/ml, susceptible; ≥8 μg/ml, resistant) were applied, and colistin, for which EUCAST clinical breakpoints for *Enterobacteriaceae* (≤2 μg/ml, susceptible; >2 μg/ml, resistant) (2) were applied. Due to moderate renal insufficiency, the patient was treated with 1 million units (MU) of colistin (polymyxin B) IVI every 8 hours combined with a 200-mg loading dose of tigecycline followed by 100 mg every 12 h IVI. Meropenem and teicoplanin were discontinued. During the course of his hospitalization, carbapenem-resistant *K. pneumoniae* was repetitively cultured from clinical specimens ($n = 17$; pus, tissue, urine, tracheal aspirates, central venous catheters, and blood), for which colistin and tigecycline were administered intermittently at the doses described above ($n = 4$ courses). Despite not being a standard procedure in this hospital, selective digestive tract decontamination (SDD) (1 MU colistin and 80 mg tobramycin every 8 h via the nasogastric [NG] tube with a 2% colistin/tobramycin paste applied every 8 h around the tonsil, NG tube, and base of the tongue) was commenced on 30 August 2011. On 16 October 2011 the patient again became hypotensive and, despite aggressive resuscitation, demised while still receiving colistin and tigecycline as described above. Blood cultures, the central venous catheter tip, and a tracheal aspirate at the time of his deterioration all grew a *K. pneumoniae* strain with sensitivities similar to those described above. The demographics and the molecular and antibiotic investigations are summarized in Table 1 and Table 2, respectively (case no. 1).

Since we recently identified *K. pneumoniae* carbapenemase (KPC-2) and New Delhi metallo-β-lactamase (NDM-1) among carbapenem-resistant *Enterobacteriaceae* (CRE), the corresponding genes were searched for by PCR as described before (3). Neither the bla_{KPC} gene nor the bla_{NDM} gene was identified; therefore,

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TABLE 1 Demographics of patients infected/colonized with OXA-48-like-producing *Enterobacteriaceae*

Case no.	Age (yr)/sex[a]	Diagnosis[b]	Comorbidity[c]	Prior hospitalization (no. of days)	Species	Source[d]	Date[e] of first isolation	Directed therapy[f]	Outcome
1	45/M	PVD and sepsis	DM, HT, RF	2	*K. pneumoniae*	Tissue	05/08/2011	Colistin and tigecycline	Deceased
2	29/M	80% burns	None	3	*K. pneumoniae*	TA	16/01/2012	Colistin and meropenem	Deceased
3	51/F	68% burns	None	28	*S. marcescens*	Tissue	19/12/2011	None (debridement)	Recovered
4	55/F	Spinal stenosis	DM, morbid obesity	12	*K. pneumoniae*	Urine	23/01/2012	None (pt already discharged)	Recovered
5	6/F	UTI	None	13	*K. pneumoniae*	Urine	05/03/2012	None (pt already discharged)	Recovered
6	82/M	CVA	RF	20	*K. pneumoniae*	TA	16/12/2012	None (pt died the following day)	Deceased
7	87/F	Collapse and resuscitation	RF	34	*K. pneumoniae*	Urine	09/02/2012	None (pt died the following day)	Deceased
8	74/M	Terminal lung CA	DM,HT, RF	12	*K. pneumoniae*	Urine	15/04/2012	None	Deceased
9	65/M	MVR and CABG	Obstructive UTI	11	*K. pneumoniae*	Urine	18/06/2012	Colistin and meropenem	Recovered

[a] M, male; F, female.
[b] PVD, peripheral vascular disease; UTI, urinary tract infection; CVA, cerebrovascular accident; CA, cancer; MVR, mitral valve replacement; CABG, coronary arterial bypass graft.
[c] DM, diabetes mellitus; HT, hypertension; RF, renal failure.
[d] TA, tracheal aspirate.
[e] Day/month/year.
[f] pt, patient.

a multiplex PCR that includes the OXA-48-like-encoding carbapenemase genes known to be widespread in North Africa was performed and gave a positive result (4). The bla_{OXA-48} gene was identified and was confirmed as an OXA-48 carbapenemase by sequencing and amino acid alignment using the primers OXA-48A (5′-TTGGTGGCATCGATTATCGG-3′) and OXA-48B (5′-GAGCACTTCTTTTGTGATGGC-3′), which amplify OXA-48 and all known OXA-48-like genes identified to date (5). This case corresponds to the first description of an OXA-48-like-producing *K. pneumoniae* isolate in South Africa (5). This patient remained colonized with the OXA-48-producing strain throughout his hospitalization (rectal swabs, $n = 7$). Confirmation of OXA-48-like-producing *K. pneumoniae* prompted us to screen for the genes among other carbapenem-nonsusceptible *Enterobacteriaceae* subsequently referred to the Ampath National Referral Laboratory. In the past 12 months, a total of 240 ertapenem-nonsusceptible isolates were screened and sequenced, and of these, 33 were found to be bla_{OXA-48} ($n = 6$) or $bla_{OXA-181}$ ($n = 27$) positive. These isolates corresponded mostly to (repeat) positive clinical cultures (and rectal carriage) from the cases described in Tables 1 and 2. In summary, the occurrence of OXA-181-producing *K. pneumoniae* in another Johannesburg hospital (case no. 2), OXA-48 in two Port Elizabeth hospitals (case no. 3 to 5), and OXA-181 in a Cape Town institution (case no. 6 to 9A and 9B) was identified. In addition, PCR and sequencing for extended-spectrum β-lactamase (ESBL)-encoding genes were performed by the method of Kiratisin et al. (6). All the *K. pneumoniae* isolates coproduced a CTX-M 15 ESBL except for case no. 3 (*Serratia marcescens*), which produced the ESBL SHV-2A (Table 2).

Case no. 9 was a hemodynamically unstable patient admitted for a mitral valve replacement and coronary arterial bypass graft surgery. The first OXA-181-producing *K. pneumoniae* strain, susceptible only to tigecycline and colistin with a MIC of 0.125 μg/ml, was cultured from a urine specimen on 18 June 2012 (Table 2, case no. 9A). Colistin monotherapy (2 MU every 12 h IVI) was administered for 5 days. In addition, he was found to be rectally colonized with the same organism, and because this organism was still present on a rectal swab on 3 July 2012 as well as for other reasons, the cardiac surgery was postponed. A second course of colistin (2 MU every 8 h IVI) and meropenem (1 g every 8 h as an extended 4-h IVI infusion) was administered in conjunction with oral SDD, using colistin and tobramycin as described above, for 22 days.

TABLE 2 Molecular studies and MICs of OXA-48-like-producing *Enterobacteriaceae*

Case no.	Species	Carbapenemase	ESBL	Other β-lactamase(s)	MHT result[a]	MIC (μg/ml)[b]												
						ERT	IPM	MEM	DOR	TZP	AMC	CTX	CAZ	FEP	AMK	CIP	TGC	COL
1	*K. pneumoniae*	OXA-48	CTX-M 15	SHV-1	+	32	16	32	32	256	256	32	64	256	**4**	32	**2**	**0.125**
2	*K. pneumoniae*	OXA-181	CTX-M 15	TEM-1, SHV-1	+	32	32	32	32	256	256	32	256	256	256	32	4	**0.0064**
3	*S. marcescens*	OXA-48	SHV-2A	TEM-1	+	32	32	32	32	256	256	32	256	256	**2**	32	4	**0.5**
4	*K. pneumoniae*	OXA-48	CTX-M 15	TEM-1, SHV-1	+	32	2	8	8	256	256	32	32	256	**2**	32	**2**	**0.25**
5	*K. pneumoniae*	OXA-48	CTX-M 15	TEM-1, SHV-1	+	2	**1**	**0.5**	**0.5**	256	256	32	16	256	256	32	**0.5**	**0.5**
6	*K. pneumoniae*	OXA-181	CTX-M 15	TEM-1, SHV-1	+	32	32	32	32	256	256	32	256	256	256	32	**2**	**0.125**
7	*K. pneumoniae*	OXA-181	CTX-M 15	TEM-1, SHV-1	+	32	32	32	32	256	256	32	256	256	256	32	**2**	**0.5**
8	*K. pneumoniae*	OXA-181	CTX-M 15	TEM-1, SHV-1	+	32	32	32	32	256	256	32	256	256	256	32	**2**	**0.25**
9A	*K. pneumoniae*	OXA-181	CTX-M 15	TEM-1, SHV-1	+	32	32	32	32	256	256	32	256	256	256	32	**2**	**0.125**
9B	*K. pneumoniae*	OXA-181	CTX-M 15	TEM-1, SHV-1	+	32	32	32	32	256	256	32	256	256	256	32	**2**	4

[a] MHT, modified Hodge test.
[b] ERT, ertapenem; IPM, imipenem; MEM, meropenem; DOR, doripenem; TZP, piperacillin-tazobactam; AMC, amoxicillin-clavulanic acid; CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime; AMK, amikacin; CIP; ciprofloxacin; TGC, tigecycline; COL, colistin. Bold type indicates susceptible MICs (1, 2).

Despite this, on 23 July 2012, OXA-181-producing *K. pneumoniae* was still present in a stool specimen, but this isolate was colistin resistant with a 5-fold increase in MIC, to 4 μg/ml (Table 2, case no. 9B).

OXA-48 was first reported in 2004 in a carbapenem-resistant *K. pneumoniae* isolate from Turkey. This resistance determinant was initially found mainly in isolates recovered from patients hospitalized in Turkey or with a link to Turkey (5, 7). Subsequently, many countries in North Africa (Morocco, Egypt, Libya, and Tunisia) as well as the Middle East (Lebanon, Israel, Sultanate of Oman, and Saudi Arabia) have reported the $bla_{\text{OXA-48}}$ gene in *K. pneumoniae*. Then, the $bla_{\text{OXA-181}}$ gene being a variant of $bla_{\text{OXA-48}}$ was identified in different members of the *Enterobacteriaceae* from patients in India (5). Several European countries have also recently reported sporadic cases and/or outbreaks of OXA-48-like producers among strains of *K. pneumoniae*, *Escherichia coli*, and *Enterobacter cloacae*, and in some of these countries, transfer of patients from North Africa, the Middle East, or India has been linked to the emergence of these organisms (5, 7–9).

What has not been reported before is the *in vivo* development of colistin resistance in a strain of OXA-181-producing *K. pneumoniae*, and this is of great concern. Resistance to colistin has sporadically been reported among KPC producers and has also been shown to occur during treatment, and outbreaks and/or interhospital spread has been documented in some instances (10, 11). Recently, *in vivo* colistin resistance was shown to evolve in NDM-1-producing *K. pneumoniae* (12). These infections were associated with high mortality, and similarly, the impact of severe infections due to colistin-resistant OXA-48-like *K. pneumoniae* could potentially be disastrous. Our case highlights three important questions. (i) Considering that colistin heteroresistance was recently reported among carbapenemase-producing *K. pneumoniae* strains, what is the optimal colistin dose not only to improve clinical outcome but to prevent selection of such resistant subpopulations (10, 11, 13)? A pharmacokinetic study in adult ICU patients with normal renal function has suggested that currently recommended doses without a loading dose resulted in significant underdosing and a delay of 48 h in achieving the breakpoint of ≥2 μg/ml (14). In this regard, Garonzik et al. (15) have recently provided a dosing model for critically ill patients with a diverse range of renal function in an attempt to obviate this problem. (ii) What is the impact of multiple and/or prolonged courses of IVI colistin? Risk factors for colistin-resistant CRE are not currently known, but in a matched, controlled study, only the use of colistin itself remained as an independent, statistically significant risk factor by multivariate analysis ($P = 0.002$; adjusted odds ratio = 7.78) (10, 11, 16). The clinical significance of colistin heteroresistance in CRE is unclear, but it appears that prior exposure increases the proportion of these subpopulations (13). Finally, (iii) what was the role of SDD with colistin in the development of colistin resistance in this case in that it not only failed to eliminate gastrointestinal carriage of class D β-lactamase (CHDL)-producing *K. pneumoniae* in two patients but also likely contributed to the selection and carriage of a colistin-resistant OXA-181-producing strain? Despite a recent meta-analysis confirming a significant reduction in rectal carriage and infections due to Gram-negative bacteria (17), oral colistin has, as in our case, previously been significantly associated with the development of colistin-resistant, ESBL-producing *K. pneumoniae* colonizers and also failed to prevent fecal colonization with any ESBL (10, 18).

It is likely that the first case of OXA-48 in South Africa that was described here occurred as a consequence of patient transfer from Egypt. Due to the fact that a carbapenem-resistant *K. pneumoniae* isolate was cultured upon admission, "search and contain" infection control strategies, as recently described by the Centers for Disease Control and Prevention (19), prevented further spread in that ICU and hospital. Epidemiological investigations could not confirm, however, that the emergence of OXA-48 and OXA-181 in the other hospitals in South Africa was related to international patient transfers, but as the focus had initially been on KPCs and NDMs, it is likely that carbapenem-hydrolyzing CHDLs had emerged and spread earlier and silently. In fact, this might be a universal phenomenon, and this possibility, together with the difficulties experienced in recognition and detection of OXA-48-like enzymes in the absence of coproduction of ESBLs and/or alterations in permeability, was recently summarized (5). In our study, coproduction of ESBLs was responsible for cephalosporin resistance while the high level of carbapenem resistance documented among the majority of our CHDLs was probably due to concomitant porin deficiency (5). Of note is case number 5, which demonstrated only ertapenem nonsusceptibility with a MIC of 2 μg/ml. Indeed, OXA-48-like carbapenemases may confer only slight increases in carbapenem MICs due to weak hydrolytic activity in the absence of associated mechanisms (5), and all such isolates necessitate further investigation. The utility of the modified Hodge test to detect OXA-48-like β-lactamase producers, including OXA-181, is supported by the fact that the test confirmed carbapenemase activity among all our strains (5, 20).

In conclusion, due to the fact that early detection is required to prevent and control dissemination of OXA-48-like carbapenemases, these organisms should be included in molecular screening, and the screening should be performed on all CRE cultured from clinical specimens. Recently it has become apparent that screening for gastrointestinal carriage of CRE may be simplified by a novel medium regardless of the ESBL status (21). As a consequence of the likelihood of coproduction of ESBLs among OXA-48-like carbapenemases and because colistin is currently the most active therapeutic option for serious infections, the use of SDD with colistin as a strategy to eradicate gastrointestinal colonization of CRE is strongly discouraged.

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