



RECTAL CULTURE SURVEILLANCE AND DEVELOPED SYSTEMIC INFECTIONS IN VANCOMYCIN-RESISTANT ENTEROCOCCI AND CARBAPENEM-RESISTANT KLEBSIELLA PNEUMONIAE CARRIERS

Çiğdem Arabacı¹, Salih Emre²

¹Medical Microbiology Laboratory, ²Clinic of Infectious Diseases and Clinical Microbiology, University of Health Sciences Okmeydani Training and Research Hospital Istanbul, Turkey

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Address for Correspondence: Çiğdem Arabacı, M.D., Medical Microbiology Laboratory, University of Health Sciences Okmeydani Training and Research Hospital, Istanbul, Turkey

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Abstract

Objectives: The increase in vancomycin-resistant enterococci (VRE) and carbapenem-resistant *Klebsiella pneumoniae* (CRKP) is a serious public health threat and a growing concern in hospitals worldwide. To determine rates of in-hospital VRE and CRKP carriage among hospitalized patients and to identify concomitant systemic VRE and CRKP infection in those with rectal colonization.

Materials and methods: A total of 3133 patients who had routine rectal swab surveillance during their hospitalization in anaesthesia intensive care unit (ICU), paediatric ICU and haematology ward over a 3-year were included in this retrospective single-center study. Rates for VRE and CRKP positivity in rectal swab cultures and systemic cultures were recorded.

Results: Rectal swab surveillance revealed VRE positivity in 335 (10.6%) and CRKP positivity in 135(4.3%) of 3133 patients assessed within the entire study period. Concomitant systemic culture positivity was significantly higher in patients with rectal CRKP carriage vs. rectal VRE carriage (57.0% vs. 17.3%, $p < 0.05$). Higher rates for rectal swap VRE (46.9% vs. 18.2% and 1.4%, respectively) and CRKP (14.1% vs. 9.0% and 0.0%, respectively) positivity was noted in haematology wards than in ICU and paediatric wards.

Conclusion: In conclusion, rates for in-hospital VRE and CRKP carriage were determined to be 10.6% and 4.3% in this rectal swab surveillance among hospitalized patients over a 3-year period. Our findings indicate higher likelihood of rectal VRE and CRKP carriage in haematology wards and ICUs than in paediatric units along with higher rate of concomitant systemic infection in patients with rectal CRKP than those with rectal VRE carriage.

Keywords: Vancomycin-resistant enterococci; carbapenem-resistant *Klebsiella pneumoniae*; hospitalization; systemic infection

1. Introduction:

Ongoing increase in vancomycin-resistant enterococci (VRE) since first described in the

mid-1980s and alarming increase in carbapenem-resistant Enterobacteriaceae (CRE), primarily carbapenem-resistant *Klebsiella pneumoniae* (CRKP) in recent years are considered a serious

public health threat and a growing concern in hospitals worldwide [1-4]. Becoming increasingly resistant to current treatment drugs, treatment infections caused by these multidrug-resistant (MDR) organisms is very challenging [5-7].

Both *Enterococcus* spp. and Enterobacteriaceae family (i.e. *Klebsiella pneumoniae*) are natural harmless inhabitants of the gastrointestinal tract [8-10]. However, depletion of commensal microbiota may result in dramatic expansion of *K. pneumoniae* in relation to their ability to thrive in and densely colonize the intestinal tract [9]. Similarly, as an opportunistic pathogen with remarkable capacity to acquire resistance to several antimicrobials and high ability for environmental adaptation [11,12], *Enterococcus* spp. are now considered one of the most common nosocomial pathogens worldwide especially due to strains of VRE [13-17].

Dense intestinal colonization in hospitalized patient is considered to induce translocation of colonizing bacteria beyond the intestinal tract in the presence of injury to mucosal barrier, which precedes the development of disseminated infections and bacteraemia by the nosocomial pathogens VRE and CRKP [18-20].

Carbapenem resistance in *K. pneumoniae* is mainly associated with acquisition of carbapenemase genes that encode for enzymes capable of hydrolysing carbapenems, including KPC-type enzymes, metallo- β -lactamases (VIM, IMP, NDM), and OXA-48 type enzymes [21-24].

Given the high mortality rates due to few antimicrobial therapy options exist for infections caused by CRKP [25,26], the U.S. Center for Disease Control and Prevention (CDC) has listed CRKP as an urgent public health threat [27], while CDC April 2013 report indicated that 30% of nosocomial responsible for 1300 deaths per year were due to VRE pathogens [28].

Accordingly, screening for epidemiology, colonization and drug resistance of these pathogens has become increasingly important for facilitating the selection and the efficacy of empirical therapy to control nosocomial

infections and to prevent the spread of antimicrobial resistance [29-32].

This retrospective study was therefore designed to determine rates of in-hospital VRE and CRKP carriage among hospitalized patients via rectal swab surveillance and to identify concomitant systemic VRE and CRKP infection in those with rectal colonization.

2. Methods and Materials

Study population

A total of 3133 patients who had routine rectal swab surveillance during their hospitalization in anaesthesia ICU (44-bed capacity), paediatric ICU (37-bed capacity) and haematology ward (10-bed capacity) of a tertiary care hospital between January 2015 and December 2017 were included in this retrospective single-center study. All patients admitted to hospital unit with high risk of nosocomial infection including ICU, paediatric ICU and haematology wards within the study period with routine rectal swab surveillance findings were included in the study.

This study was approved by Ministry of Health Okmeydanı Training and Research Hospital's local Ethics Committee. Informed consent was obtained by all patients.

Rectal swab surveillance

Rectal swab surveillance has been routinely performed in our hospital on a weekly basis in ICU patients, on a monthly basis (weekly in those with rectal carriage) in paediatric ICU patients, and twice weekly in haematology inpatients. Rectal swab surveillance was indicated in case of detection of VRE or CRKP positivity in any culture specimen among patients hospitalized in other units.

Rectal specimens were collected using premoistened double-headed rayon swabs (BBL Culture Swab; Becton, Dickinson, Sparks, MD) and placed in liquid Amies medium for transport to the microbiology laboratory for processing.

Culturing was performed with inoculation of rayon swabs directly onto ChromID VRE (bioMérieux) and CHROMagar KPC (CHROMagar)

plates that were freshly prepared on the day of the study according to the manufacturer's instructions and poured into 90-mm-diameter petri dishes via agar dilution method. The plates were incubated 48 hours at 37°C in ambient air and then examined for growth of *Klebsiella* spp. [metallic blue (*K. pneumoniae*) colonies of gram negative bacteria in CHROMagar] and *Enterobacter* spp. [purple (*Enterococcus faecium*) or blue-green (*Enterococcus faecalis*) colonies of gram positive bacteria on ChromID VRE agar].

Bacteria that were identified via Phoenix 100 (Phoenix Becton Dickinson ID, ABD) automatized system were analysed for antibiotic sensitivity using EUCAST criteria. Accordingly, *E. faecium* and *E. faecalis* with vancomycin minimum inhibitory concentration (MIC) > 4µg/mL were considered to be VRE, while *K. pneumoniae* with imipenem and meropenem MIC >8µg/mL were considered to be CRKP.

Study parameters

Rates for VRE and CRKP positivity in rectal swab cultures as well as in other systemic culture samples (i.e. urine, blood, catheter blood, catheter tip, cerebrospinal fluid, peritoneal fluid samples) during follow up of patients were recorded. Data on antibiotic treatment in patients with VRE positive urine cultures were retrieved from the medical records and from consultation notes of infectious diseases department.

Statistical analysis

Descriptive statistics including percentages were reported using IBM SPSS Statistics for Windows, version 22.0 (IBM Corp., Armonk, NY). Chi-Square test was used to analyse systemic infection rates in relation to rectal VRE and CRKP carriage status. p<0.05 was considered statistically significant.

3. Results

Rectal swab surveillance findings according to study period

Overall, 6198 rectal swab samples in 3133 patients were analysed over a 3-year period including 1604 samples in 833 patients in 2015, 2062 samples in 1035 patients in 2016 and 2532 samples in 1215 patients in 2017.

Rectal swab surveillance revealed VRE positivity in 335 (10.6%) and CRKP positivity in 135(4.3%) of 3133 patients assessed within the entire study period. Concomitant systemic culture positivity was significantly higher in patients with rectal CRKP carriage vs. rectal VRE carriage (in 77 of 135 (57.0%) vs. 58 of 335 (17.3%) patients, p<0.05). In 79 (2.5%) patients, there was rectal swab positivity for both VRE and CRKP (Table 1).

In years 2015, 2016 and 2017, rectal VRE carriage rates were 10.4%, 10.6% and 10.9%, whereas rectal CRKP carriage rates were 5.2%, 4.6% and 3.3%, respectively (Table 1).

Table 1: Rectal swab surveillance findings and concomitant systemic culture positivity according to study period

		2015-2017 (n=3133)	2015 (n= 883)	2016 (n=1035)	2017 (n=1215)
Culture positivity, n (%)					
VRE	Rectal swab	335(10.6)	92(10.4)	110(10.6)	133(10.9)
	Plus systemic	58(17.3)	13(14.1)	19(17.2)	26(19.5)
CRKP	Rectal swab	135(4.3)	46(5.2)	48(4.6)	41(3.3)
	Plus systemic	77(57.0)*	23(50.0)	26(54.1)	28(68.2)
Rectal swab VRE/CRKP		79(2.5)	28(3.1)	26(2.5)	25(2.0)
VRE: Vancomycin-resistant enterococci CRKP: Carbapenem-resistant <i>Klebsiella pneumoniae</i> *p<0.05; compared to systemic culture positivity in the VRE group (Chi-Square test)					

Rectal swab surveillance findings according to hospital unit

Paediatric ICU, anaesthesia ICU and haematology patients composed 54.6%, 38.2% and 5.6% of overall study population with higher rates for rectal VRE carriage (46.9% vs. 18.2% and 1.4%, respectively) and rectal CRKP carriage (14.1% vs. 9.0% and 0.0%, respectively) in haematology wards than in adult and paediatric ICU wards (Table 2).

In years 2015, 2016 and 2017 among haematology patients, rectal swap culture

positivity rates were 61.9%, 36.5% and 40.3% for VRE, and were 25.4%, 9.6% and 6.5% for CRKP, respectively (Table 2).

In years 2015, 2016 and 2017 among adult ICU patients, rectal swap culture positivity rates were 16.2%, 17.5% and 19.9% for VRE, whereas were 10.2%, 9.5% and 7.5% for CRKP, respectively. None of the paediatric patients had rectal swap culture positivity for CRKP positivity during the study period (Table 2).

Table 2: Rectal swab surveillance findings and concomitant systemic culture positivity according to hospital unit

	2015-2017 (n=3133)			2015 (n= 883)			2016 (n=1035)			2017(n=1215)		
	P-ICU	A-ICU	Haemato	P-ICU	A-ICU	Haemato	P-ICU	A-ICU	Haemato	P-ICU	A-ICU	Haemato
Patients, n (%)	1711 (54.6)	1198 (38.2)	177 (5.6)	530 (60.0)	271 (30.7)	63 (7.1)	519 (50.1)	450 (43.5)	52 (5.0)	662 (54.5)	477 (39.3)	62 (5.1)
VRE (+), n (%)												
Rectal swab	24 (1.4)	218 (18.2)	83 (46.9)	6 (1.1)	44 (16.2)	39 (61.9)	7 (1.3)	79 (17.5)	19 (36.5)	11 (1.7)	95 (19.9)	25 (40.3)
Plus systemic	2 (0.1)	46 (3.8)	8(4.5)	0(0.0)	8 (3.0)	3 (4.3)	1 (0.1)	15 (3.3)	1 (1.9)	1 (0.1)	23 (4.8)	4 (6.5)
CRKP (+), n (%)												
Rectal swab	0 (0.0)	108 (9.0)	25 (14.1)	0 (0.0)	29 (10.2)	16 (25.4)	0 (0.0)	43 (9.5)	5 (9.6)	0 (0.0)	36 (7.5)	4 (6.5)
Plus systemic	0 (0.0)	64 (5.3)	12 (6.8)	0 (0.0)	17 (6.3)	6 (9.5)	0 (0.0)	22 (4.9)	4 (7.7)	0 (0.0)	25 (5.2)	2 (3.2)

P-ICU: Paediatric intensive care unit; A-ICU: anaesthesia intensive care unit; Haemato: haematology;

VRE: Vancomycin-resistant enterococci; CRKP: Carbapenem-resistant Klebsiella pneumoniae

Sites of positive systemic culture specimens in patients with VRE and CRKP carriage

In patients with VRE carriage and concomitant systemic culture positivity (n=58), culture sites with VRE growth involved urine (n=47), blood (n=8) and catheter blood (n=8) in most cases. In those with CRKP carriage and systemic infection (n=77), positive culture sites involved blood

(n=44), catheter blood (n=33), urine (n=27), tracheal aspirate (n=17) and catheter tip (n=10) (Table 3).

Treatment for VRE was administered in 30 of 58 patients with rectal swab and systemic culture VRE positivity including 23 of 47 cases with positive urine cultures (Table 3).

Table 3: Sites of positive systemic culture specimens in patients with VRE and CRKP-positive rectal swap cultures

Sites of specimens	2015-2017 (n=3133)		2015 (n= 883)		2016 (n=1035)		2017(n=1215)	
	VRE (n=58)	CRKP (n=77)	VRE (n=13)	CRKP (n=23)	VRE (n=19)	CRKP (n=26)	VRE (n=26)	CRKP (n=28)
Urine	47	27	10	9	17	9	20	9
Blood	8	44	3	17	1	17	4	10
Catheter blood	8	33	1	7	2	18	5	8
Peritoneal fluid	1	2				2	1	
Wound site	1	2		1		1	1	
Catheter tip		10		1		6		3
Tracheal aspirate		17		1		7		9
Tissue		4				1		3
CSF		1						1
Abscess		1						1
VRE-treatment								
Total	30/58		9/13		8/19		13/26	
In urine culture positive cases	23/47		6/10		7/17		10/20	

VRE: Vancomycin-resistant enterococci; CRKP: Carbapenem-resistant Klebsiella pneumoniae

4. Discussion

This retrospective analysis of 6198 rectal swab samples from 3133 inpatients over a 3-year period revealed the prevalence of rectal VRE carriage to be 10.6% and prevalence of rectal CRKP carriage to be 4.3%, along with higher likelihood of concomitant systemic bacterial growth in case of rectal CRKP (57.0%) vs. VRE (17.3%) carriage. In years 2015, 2016 and 2017, rectal swap positivity rates were 10.4%, 10.6% and 10.9% for VRE, whereas were 5.2%, 4.6% and 3.3% for CRKP, respectively. The overall prevalence of carbapenem resistant Klebsiella spp. isolates causing hospital-acquired infections was reported to be approximately 12% between 2009 and 2010 in U.S. hospitals [33,34], while epidemiological situation for CRKP had worsened worldwide since 2010 with spread in European hospitals, particularly in the Mediterranean area [26].

Data from the European Antimicrobial Resistance Surveillance Network (EARS-Net) by the

European CDC (ECDC) showed an increasing prevalence of CRKP isolates between 2005 and 2010 in Europe, with increase in the number of countries with $\geq 1\%$ CRKP isolates from 2 in 2005 (Greece, 27.8%; Germany, 3.1%) to 5 in 2010 (Greece, 49.8%; Cyprus, 16.4%; Italy, 12.5%; Hungary, 5.9%; Portugal, 2.2%) [35,36].

According to the European survey of carbapenemase-producing Enterobacteriaceae (EuSCAPE) project launched by ECDC in 2012, transition to a higher epidemiological stage was reported in 17 countries of 31 countries that participated in both 2010 and 2013 assessments [37]. However, from 2013 to 2015, the epidemiological situation of CRE worsened, in particular with the rapid spread of OXA-48- and NDM-producing Enterobacteriaceae, alongside the increase in the number of countries reporting inter-regional spread of or an endemic situation for CRE from 6/38 countries in 2013 to 13/38 countries in 2015 [37,38]. Although, Turkey was amongst the three countries with no

case of CRKP reported in 2014–2015, epidemiological situation has worsened in Turkey since 2015, which has changed from single outbreak before 2015 to an endemic situation in 2015 [38].

Hence, CRKP rates in our cohort seem consistent with the rapid dissemination of CRKP with a considerably increasing rate over the past few years in Turkey, alongside the growing global prevalence [38-40].

In addition, while OXA-48-producing Enterobacteriaceae are endemic and still the predominant mechanism of resistance in Turkey, since 2013, an increasing number of reports have demonstrated the emergence of other types of carbapenem resistance (e.g. NDM-1- and KPC-producing Enterobacteriaceae) [38,41-46].

In our cohort, the rates for rectal VRE and CRKP carriage were highest among haematology inpatients (46.9% and 14.1%, respectively) and followed by anaesthesia ICU patients (18.2% and 9.0%, respectively), while none of the paediatric ICU patients had rectal CRKP carriage and rectal VRE carriage was noted only in 1.4% of paediatric cases. Hence, our findings indicate higher likelihood of rectal VRE and CRKP carriage in immunosuppressive or critically ill adult inpatients.

This seems consistent with consideration of high risk immunocompromised populations such as ICU, solid organ transplant (SOT), haematological malignancy and stem cell transplant patients to be at risk of CRKP with poorer prognosis [26]. Similarly, host characteristics (immunosuppression, neutropenia, and renal insufficiency), hospital factors (extended length of stay, admission to an ICU or oncology ward, proximity to a VRE-colonized patient), and antimicrobial use have also been reported to be risk factors for VRE colonization [16,47-49].

Our findings on rectal VRE carriage are also consistent with data on VRE carriage from Turkey, as reported to be 12.9% (n=486) in 3772 rectal swabs in 2012-2017 [31], supporting the consideration of colonization and infection with

VRE as an increasingly common problem in hospitals [31,49].

Yearly distribution of colonization rates from 2015 to 2017 in our cohort emphasizes a gradually decreasing trend for CRKP in anaesthesia ICU (10.2% to 7.5%) and haematology (25.4% to 6.5%) wards and for VRE in haematology wards (61.9% to 40.3%), while an increasing trend for VRE was noted in paediatric ICU (1.1% to 1.7%) and anaesthesia ICU (16.2% to 19.9%).

Although not evident in our paediatric ICU patients, past studies from Turkey revealed that asymptomatic colonization with CRKP in paediatric units should alert health care providers about forthcoming CRKP infection [50,51]. CRKP colonization rates were reported to be 3.6% and 2.6% in paediatric and neonatal ICUs throughout a five-year period (2010-2014), alongside development of systemic CRKP infection in 39.1% of paediatric and 18.1% of neonatal patients [50]. CRKP colonization via rectal swabs culture was reported in 83(29.5%) of 281 paediatric patients, along with development of CRKP infection in 3(3.4%) of all colonized patients, including bacteraemia, urinary tract infection and soft tissue infection [51].

Nonetheless, VRE rates in our paediatric patients are consistent with findings from a retrospective cohort of 260 children with VRE colonization (62.3% from ICU and 33.0% from haematology-oncology department) detected during routine surveillance between 2009 and 2012 in Turkey, which indicated VRE bacteraemia only in 3(1.55%) patients, particularly in those with immunosuppression [52].

In a past study from Turkey with 185 adult patients hospitalized in hematopoietic stem cell transplantation units over a period of 8 months, analysis of rectal swab samples revealed carbapenem-resistant gram-negative bacilli in 21(11.4%) patients and development of febrile neutropenia in 9(56.2%) of the 21 colonized patients [53]. Similarly, in a prospective 39-month study of 498 ICU patients, rectal

colonization of CRKP was reported in 226 patients, while a CRKP blood stream infection was noted in 48 of 226 patients within a median 4 days from the colonization [54].

Our findings revealed higher likelihood of concomitant systemic infection in rectal CRKP carriage as compared with rectal VRE carriage. Moreover, while urine culture positivity (in 47 of 58 patients) was the most common systemic involvement in patients with rectal VRE carriage, positive blood cultures (n=44) and positive catheter blood cultures (n=33) were more frequent than positive urine cultures (n=27) in patients with rectal CRKP carriage.

In a systematic review and meta-analysis, mortality rates were reported to be higher (42.14% vs. 21.16%) in patients infected with CRKP than those infected with carbapenem-susceptible *K. pneumoniae* (CSKP), particularly in association with blood stream infection (BSI), ICU admission or SOT rather than the urinary tract infection (UTI) [55]. Given that mortality rate was higher in patients with BSI, ICU admission or SOT as compared to pooled mortality, while UTI was associated with mortality rates lower than pooled or CSKP-related mortality, authors suggested the likelihood of a close relationship between underlying illness/comorbidities with survival [55].

Hence, given the higher risk of systemic involvement and higher likelihood of systemic infections other than UTI in rectal CRKP carriage than in rectal VRE carriage, our findings emphasize the importance of rectal surveillance among hospitalized patients for rectal CRKP as well as search for systemic infection foci in those with rectal CRKP colonization.

Likewise, in a cohort of 36 cases with BSI due to CRE from Turkey, microbiological eradication and clinical improvement on day 7 were found to be two major indicators of 28-day survival [56]. Hence, authors emphasized that rectal screening offers the advantage of earlier recognition and prompt empirical treatment [56].

Similarly, active screening for VRE carriage via rectal swabs is also considered helpful for rapid and accurate identification and to prevent nosocomial spread of VRE, given the association of VRE colonization with an increased risk of developing infections (i.e. BSI) [30].

Alongside the limited repertoire of antibiotics available to treat CRKP, presence of carbapenemases as accompanied with other resistance traits to aminoglycosides and fluoroquinolones leads to extensively drug-resistant (XDR) or pandrug-resistant (PDR) condition with few or no effective treatment options [1,36,57,58]. This increases the risk of CRE to disseminate rapidly not only within hospitals, but also beyond the hospital into the community [1,36,58].

Accordingly, good surveillance and active screening of high-risk patients, strict implementation of targeted infection control measures and prudent use of antimicrobials are considered key elements for efficient monitoring and controlling of the spread of CRE, CRKP in particular [1,36,38,55,59].

In past studies from Turkey, 75.5% to 86.5% of suspected carbapenemase-positive isolates were identified to be *K. pneumoniae*, with at least one resistance to imipenem (52.1-59.5%), meropenem (52.9-73.2%) and ertapenem (100%) [60].

Accordingly, as all of the OXA-48 producing isolates were found to be resistant to ertapenem, ertapenem is considered the most sensitive agent in screening carbapenemases in areas with high OXA-48 prevalence [60]

In our cohort, concomitant rectal colonization by VRE and CRKP was evident in 79(2.5%) patients. Notably, VRE and CRKP are considered likely to localize to the same intestinal regions and maintain independent growth and persistence in the gut without impairing each other's colonization, while they differ with respect to stimulation and invasion of the colonic mucus layer [20].

Certain limitations to this study should be considered. First inherent to its retrospective design, establishing the temporality between cause and effect is limited. Second, being a single-center study, our findings on antimicrobial susceptibility may not reflect the situation at the regional level. Third, lack of data on specific antibiotic resistance genes and enzymes with molecular or genetic tests or clonal dissemination of isolates is another limitation which otherwise would extend the knowledge achieved in the current study.

5. Conclusions

In conclusion, rates for in-hospital VRE and CRKP carriage were determined to be 10.6% and 4.3% in this rectal swab surveillance among hospitalized patients over a 3-year period. Our findings indicate higher likelihood of rectal VRE and CRKP carriage in haematology wards and ICUs than in paediatric units, emphasizing the higher risk for colonization by VRE and CRKP in immunosuppressive or critically ill adult patients. Concomitant systemic infection, BSI in particular, was more common with rectal CRKP than with rectal VRE carriage. Hence, our findings emphasize the potential role of rectal screening for CRKP high-risk patients in earlier recognition of colonization and thus prompt empirical treatment in those with or at risk of systemic infection.

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