Table 1. Cumulative Days of Antipseudomonal β-Lactam Antibiotic Exposure and New Resistance Development

Cumulative Days of Antipseudomonal Exposure	No. of Patients	New Resistance Events, No. (%)	Hazard Ratio (95% Confidence Interval)
1–3	1,816	38 (2.09)	1.00 (reference)
4–6	1,632	85 (5.21)	1.01 (0.93–1.10)
7–9	1,249	98 (7.85)	1.85 (1.69–2.02)
10–12	709	66 (9.31)	2.93 (2.66–3.24)
13–15	474	44 (9.28)	3.94 (3.54–4.39)
16–18	326	30 (9.20)	6.29 (5.62–7.04)
19–21	234	27 (11.5)	7.05 (6.19–8.02)
≥22	678	56 (8.3)	8.52 (7.62–9.53)

Discussion

Our retrospective cohort study showed the associated rise in the risk of new resistance emergence with increasing duration of antipseudomonal β-lactam antibiotic exposure in the critically ill does not appear to exhibit a "ceiling effect" as the cumulative duration of exposure increases. This finding is important because it suggests that the risk of new resistance will continue to increase as the duration of exposure increases, regardless of how long the patient has been on antimicrobial therapy.

Recent estimates showing antibiotic resistances accounting for >2.8 million infections and >35,000 death per year in the United States highlight the need to understand and prevent resistance development.[2] Minimizing durations of antimicrobial therapy is becoming a pillar of antimicrobial stewardship; however, studies evaluating optimal durations are lacking, and many guideline recommendations for duration of therapy continue to rely on expert opinion which may result in longer than necessary exposures.[5–7] Our study further highlights the need for further studies evaluating optimal durations for various types of infections as well as studies regarding strategies to limit antimicrobial exposure to the shortest effective duration.

Acknowledgments. None.

Financial support. Marin Kollef's efforts were supported by the Barnes-Jewish Hospital Foundation.

Conflicts of interest. All authors report no conflicts of interest relevant to this article.

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Silent clonal spread of vancomycin-resistant *Enterococcus faecalis* ST6 and ST525 colonizing patients at hospital admission in Natal, Brazil

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To the Editor—Infections and gut colonization with vancomycin-resistant enterococci (VRE) have been increasingly reported in hospitalized patients from different regions of Brazil, where difficulties in controlling VRE colonization have been noted.[1-4] Patient colonization with VRE is a major risk factor for developing subsequent infections with these strains.[1,5] The first VRE description dates from 2011 (M. Celeste Melo, personnel data), and VRE infections among hospitalized patients from Natal city (northeastern Brazil) remain low, contrasting with the high rates of VRE infection and colonization of the patients in the southern and southeastern regions, where they have occurred since 1998.[1-3] For early recognition of silent interhospital VRE transmission through colonized patients as in other parts of Brazil, we aimed to search and characterize VRE colonization strains from patients known to have a previously history of hospitalization.

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Cite this article: de Oliveira ES, *et al.* (2020). Silent clonal spread of vancomycin-resistant *Enterococcus faecalis* ST6 and ST525 colonizing patients at hospital admission in Natal, Brazil. *Infection Control & Hospital Epidemiology*, 41: 485–487, https://doi.org/10.1017/ice.2020.24

https://doi.org/10.1017/ice.2020.24 Published online by Cambridge University Press

Table 1. Genetic Characterization of 18 Representative *Enterococcus faecalis* With Different Epidemiological Backgrounds

						Acquired Antibiotic Resistance Genes						Virulence-Associated Genes						
PFGE	MLST	Hospital	Patient[a]	Sample	Date	*vanA*	*erm(B)*	*tet(M)*	*ac(6')-Ie-aph(2")-Ia*	*aph(3')-III*	*ant(6)-Ia*	*gelE-fsrB*	*cyl*	*ace*	*agg*	*efaAfs*	*ebpABC*	*srtA*
A	ST525	HB	**17**	Perianal swab	13.09.2015	+	+	+	+	–	–	+	+	+	–	+	+	+
A	ST525	HA	22	Perianal swab	11.10.2015	+	+	+	+	–	–	+	+	+	–	+	+	+
A	ST525	HA	18	Catheter tip	20.10.2015	+	+	+	+	–	–	+	+	+	–	+	+	+
A	ST525	Home (OP)	28	Perianal swab	08.01.2016	+	+	+	–	–	–	+	+	+	–	+	+	+
A	ST525	HF	36	Perianal swab	09.03.2016	+	+	+	+	–	–	+	+	+	–	+	+	+
A	ST525	Home (OP)	46	Ulcer secretion	29.11.2016	+	+	+	+	–	–	+	+	+	–	+	+	+
A	ST6	HC	3	Urine	01.06.2015	+	+	+	+	–	–	+	+	+	+	+	+	+
A	ST6	HA	11	Perianal swab	30.06.2015	+	+	+	+	+	+	+	+	+	+	+	+	+
B	ST525	HE	2	Perianal swab	18.05.2015	+	+	+	+	–	–	+	+	+	+	+	+	+
B	ST525	HF	10	Ocular secretion	26.06.2015	+	+	+	+	–	–	+	+	+	+	+	+	+
B	ST525	HC	38	Perianal swab	12.03.2016	+	+	+	+	–	–	+	+	+	–	+	+	+
B	ST525	HB	41	Urine	08.04.2016	+	+	+	+	–	–	+	+	+	–	+	+	+
B	ST525	HB	**17**	Perianal swab	30.11.2016	+	–	+	+	–	–	+	+	+	–	+	+	+
B	ST525	HC	47	Perianal swab	12.12.2016	+	+	+	+	–	–	+	+	+	+	+	+	+
B	ST525	HE	26	Perianal swab	unknown	+	+	+	+	–	–	+	+	+	+	+	+	+
B	ST6	HB	**9**	Bone fragment	25.06.2015	+	+	+	+	+	+	+	+	+	+	+	+	+
B	ST6	HC	**9**	Perianal swab	02.07.2015	+	+	+	+	+	+	+	+	+	+	+	+	+
C	ST6	HA	45	Perianal swab	unknown	+	+	+	+	+	+	+	+	+	+	+	+	+

Note. PFGE, pulsed-field-gel-electrophoresis; MLST, multilocus sequence typing; OP, outpatient
[a]When the same patient carried VRE in >1 occasion, the data are shown in bold.

Glycopetides
Macrolides
Tetracyclines
Aminoglycosides
Secreted factor for tissue damage
Adhesins and biofilm-associated genes

From January 2015 to December 2016, 55 VRE isolates were recovered (chromID-VRE) from perianal swabs of 44 inpatients at 5 hospitals (range, 36–257 beds) in Natal (public hospitals A, B, and F; public–private hospital C, or private hospital E) during hospital admission and of 3 outpatients when receiving domiciliary nursing care at their own home. Most of these patients had previous hospitalization episodes. Six additional VRE were collected from different clinical specimens during the hospitalizations (in hospitals A, B, C, D, and F) of 6 patients (2 in common with those from the cohort of 44 patients). Species identification and search of antibiotic resistance, virulence, and plasmid replicase genes were conducted using polymerase chain reaction testing (PCR) in isolates representative of different clones and epidemiological contexts.[6,7] Antibiotic susceptibility was assessed using disk diffusion (ie, for ampicillin, tetracycline, chloramphenicol, gentamicin, erythromycin, and streptomycin), Etest (vancomycin), agar dilution (teicoplanin), or broth microdilution (linezolid) following the European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints (http://www.eucast.org/clinical_breakpoints/) or, when not possible, Clinical and Laboratory Standards Institute (CLSI) breakpoints. Clonal relationships were firstly evaluated by *SmaI* pulsed-field gel electrophoresis (PFGE) in the 61 VRE and by multilocus sequence typing (MLST) in 18 *Enterococcus faecalis* (*Efs*) (ie, different PFGE types, patients, and hospitals).[6]

The VRE were identified as *Efs* (n = 60) and *E. faecium* (n = 1), all carrying *vanA*. In this study, we only proceeded with the *Efs*; all were multidrug resistant (MDR) and exhibited resistance to vancomycin (range, 16 to >256 mg/L), teicoplanin (range, 4–64 mg/L), ciprofloxacin, tetracycline, and erythromycin. Most were also resistant to gentamicin (93%) and streptomycin (42%). PFGE established 3 pulsotypes among the 60 VR*Efs*: 30 for pulsotype A, 29 for pulsotype B, and 1 for pulsotype C. The dominant pulsotypes A and B were dispersed among all institutions and were detected throughout the study period. Representative VR*Efs* from pulsotypes A and B were identified as sequence type 6 (ST6; n = 4: 2 of pulsotype A and 2 of pulsotype B) or ST525 (n = 13: 6 of pulsotype A and 7 of pulsotype B). Pulsotype-C was also an ST6 (Table 1). Examples of *Efs* strains showing the same or similar pulsotypes but clustering in different sequence types have previously been described.[8] ST6 is dispersed worldwide mostly in association with nosocomial infections, whereas ST525 has only been documented in Brazil southeastern regions (VR*Efs* linezolid-resistant infections; https://pubmlst.org),[4,9] suggesting the dispersion of relevant strains of such lineage in this country. A patient was colonized with both ST525 A and B clones collected 14 months apart. The same PFGE-B/ST6 *Efs* was associated with 2 isolates from the same patient (bone fragment and colonization) obtained 7 days apart in different hospitals (Table 1). Moreover, *vanA* plasmid replicases from the 18 VR*Efs* were identical (rep_9 from pheromone-responsive pTEF2/pAD1 plasmids) and known to transfer highly efficiently, which could have enhanced vancomycin-resistance spread also by horizontal gene transfer. Among the 18 VR*Efs*, all carried *vanA*, *erm(B)*, *tet(M)*, *aac(6′)-aph(2″)*, whereas *aph(3′)-III*, and *ant(6)-Ia* were variable. Putative virulence factors included adhesins, biofilm-associated genes, and survival genes (eg, *ace*, *gelE*, and *agg/cyl*) previously associated with dominant lineages of human infections (Table 1).[10]

In summary, patients with a previous history of hospitalization and who had been admitted to different hospitals of Natal were colonized with 2 VR*Efs* clonal lineages with potential to cause infection, one closely related to global ST6-VR*Efs* and the other more region specific (ST525).[4,9] The detection of several *Efs* strains with the same PFGE type associated with different sequence types (via MLST) highlights both the limited accuracy of these methods to identify identical *Efs* strains and the need to use methods with a higher resolution to better follow transmission events between patients and institutions. The identification of 2 specific VR*Efs* clones is reminiscent of the emergence of VRE in other regions of Brazil in previous years, where VR*Efs* strains are now being replaced by dominant vancomycin-resistant hospital-associated *E. faecium*.[2,3] Our results highlight the need for better hygiene measures, systematic colonization survey of transferred patients, patient isolation, and antimicrobial stewardship to prevent future epidemic and endemic scenarios associated with infection in hospitals from Natal. Of special concern is the transfer of such VRE to the community level by colonized patients with domiciliary nursing, which represents another potential level of transmission requiring additional strategies to control their spread.

Acknowledgments. None.

Financial support. This work was supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and by funding from the Applied Molecular Biosciences Unit - UCIBIO which is financed by national funds from FCT (UIDB/04378/2020). A.R.F. gratefully acknowledges the junior research position funded by FCT/MCTES through national funds (grant no. CEECIND/02268/2017, Individual Call to Scientific Employment Stimulus 2017).

Conflicts of interest. All authors report no conflicts of interest relevant to this article.

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https://doi.org/10.1017/ice.2020.24 Published online by Cambridge University Press