plaque microbiota was 4.7×10^6 CFU/mL (range 6.5×10^5 to 7.8×10^7 CFU/mL) and the median viable count of *Veillonella* spp. was 1.3×10^5 CFU/mL (range <10 CFU/mL to 5.8×10^6 CFU/mL). The proportion of the cultivable plaque microbiota that comprised *Veillonella* spp. ranged from <0.001% in two subjects to a maximum of 11.6%, with a median of 4.2%.

In total, 158 *Veillonella* isolates were obtained from the 58 subjects. The majority of these isolates were identified as *Veillonella parvula* (47.5%), followed by *Veillonella dispar* (40.5%) and *Veillonella atypica* (9.5%) (Table 1). Owing to 16S rRNA gene similarity, four isolates (2.5%) could not be identified to species level.

Interestingly, β-lactam resistance was commonly demonstrated in the 158 *Veillonella* isolates, with 63.9% penicillin resistance (MIC ≥ 8 mg/L) and 39.2% ampicillin resistance (MIC ≥ 8 mg/L) (Table 1). The highest rate of penicillin resistance was exhibited by *V. dispar* (73.4%), and *V. atypica* had the highest rate of ampicillin resistance (46.7%). None of the antibiotic-resistant isolates produced a β-lactamase as determined by the nitrocefin test.

Previous studies have suggested that *Veillonella* spp. are generally susceptible to penicillin and ampicillin and it has been suggested that use of penicillin or other β-lactam antibiotics would be the treatment of choice for *Veillonella* infection [1,4]. However, the results of this study demonstrated high rates of penicillin resistance, which concurs with the results of a Spanish study that reported >80% of *Veillonella* spp. as non-susceptible to penicillin, although it was in a smaller population of 40 isolates [5]. A study carried out in 1976 reported 5% of *Veillonella* isolates as ampicillin-resistant [4]; in the current study, 46.7% of the *V. atypica* isolates were ampicillin-resistant. These data suggest that *Veillonella* spp. have altered from a genus that was predominantly sensitive to penicillin and ampicillin to one that is now frequently resistant to these antibiotics.

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Extended-spectrum β-lactamases in *Escherichia coli* and *Klebsiella pneumoniae* over 18 years: effect of different systems for eliminating duplicates

Sir,

The increase in resistance of Gram-negative bacilli is a serious public health problem, and elimination of duplicates plays a very important role in quantifying this increase [1]. We recently studied the effect of different duplicate elimination criteria on the evolution of fluoroquinolone resistance in *Escherichia coli* and *Klebsiella pneumoniae* in the General Hospital in Elche, a 450-bed hospital in the southeast of Spain, over an 18-year period [2]. The aim of this study was to apply the same criteria when determining the evolution of extended-spectrum β-lactamase (ESBL)-producing isolates. The criteria used are [2]:

- total criterion: taking into account all the clinical isolates obtained;
- Clinical and Laboratory Standards Institute (CLSI) criterion: taking into account only the first isolate from each patient;
- time criterion: taking into account the first isolate and the samples isolated after 7 days or 30 days; and
- European Antimicrobial Resistance Surveillance System (EARSS) criterion: taking into account the first isolate and the isolates that exhibited a change in antibiotic susceptibility to some antibiotics [3].

Antibiotic susceptibility was studied by microdilution using a Wider semiautomatic system (Soria Melguizo, Madrid, Spain). All ESBL-producing isolates were confirmed by Etest (AB BIODISK, Solna, Sweden) according to CLSI methods [4].

Different prevalences of ESBL-producing *E. coli* isolates were found when the criteria recommended by the CLSI and EARSS were compared (Table 1). An absolute difference of 194 isolates [31.09%, 95% confidence interval (CI) 27.38–34.80%] was found between EARSS and CLSI criteria (624 ESBL-producing isolates were identified according to EARSS versus 430 isolates according to CLSI). The difference increased in the outpatient setting to 39.55% (95% CI 34.86–44.23%; 440 vs. 266), whereas in hospitalised patients it was reduced to 10.87% (95% CI 6.10–15.64%; 184 vs. 164) ($P < 0.01$).

Klebsiella pneumoniae prevalences are shown in Table 1. An absolute difference of 32 ESBL-producing isolates (47.76%, 95% CI 35.05–60.47) was found (67 isolates were identified according to EARSS versus 35 according to CLSI). Differences between patients admitted to hospital and those who were not were not statistically significant ($P = 0.72$) [51.52% (95% CI 32.95–70.08) vs. 44.12% (95% CI 25.96–62.28), respectively] (Table 1).

Treatment of infections caused by ESBL-producing isolates is very different to that recommended in the empirical management of the main infectious conditions. Therefore, when drawing up local

Table 1
Extended-spectrum β-lactamases (ESBLs) in *Escherichia coli* and *Klebsiella pneumoniae:* effect of different criteria for elimination of duplicates in samples from hospitalised and non-hospitalised patients.

Criterion	Total isolates			Inpatients			Outpatients		
	N	ESBL-producing		*N*	ESBL-producing		*N*	ESBL-producing	
		n	% (95% CI)		*n*	% (95% CI)		*n*	% (95% CI)
Escherichia coli									
Total	19 513	1030	5.3 (5.0–5.6)	5282	477	9.0 (8.2–9.8)	14 231	553	3.9 (3.6–4.2)
CLSI	13 552	430	3.2 (2.9–3.5)	3104	164	5.3 (4.5–6.1)	10 448	266	2.5 (2.2–2.9)
7-day	17 574	723	4.1 (3.8–4.4)	4352	181	4.2 (3.6–4.8)	13 222	542	4.1 (3.8–4.4)
30-day	16 902	679	4.0 (3.7–4.3)	4086	160	3.9 (3.3–4.5)	12 816	519	4.0 (3.7–4.4)
EARSS	16 106	624	3.9 (3.6–4.2)	4182	184	4.4 (3.8–5.0)	11 924	440	3.7 (3.3–4.0)
Klebsiella pneumoniae									
Total	4618	79	1.7 (1.3–2.1)	1275	37	2.9 (1.9–3.9)	3343	42	1.3 (0.9–1.6)
CLSI	2493	35	1.4 (0.9–1.9)	865	16	1.8 (0.9–2.8)	1628	19	1.2 (0.6–1.7)
7-day	4213	68	1.6 (1.2–2.0)	1242	30	2.4 (1.5–3.3)	2971	38	1.3 (0.9–1.7)
30-day	4081	66	1.6 (1.2–2.0)	1207	29	2.4 (1.5–3.3)	2874	37	1.3 (0.9–1.7)
EARSS	3810	67	1.8 (1.3–2.2)	1124	33	2.9 (1.9–4.0)	2686	34	1.3 (0.8–1.7)

CLSI, Clinical and Laboratory Standards Institute; EARSS, European Antimicrobial Resistance Surveillance System.

guidelines for empirical therapy, accurate knowledge of the situation in each area is vital to ensure the usefulness of such guidelines [2], especially if we bear in mind that incorrect empirical treatment of these infections has been associated with a significant increase in early mortality and overall mortality in patients with ESBL-producing *E. coli* infections [5].

The prevalence of ESBLs in Europe has been reported to be higher than in the USA; however, there are important differences between European countries associated with the spread of mobile genetic elements, mainly epidemic plasmids, and the dispersion of specific clones [6], thus making it essential to understand the real situation in each setting.

This study shows that the CLSI duplicate elimination criterion of ESBL-producing isolates in *E. coli* and *K. pneumoniae* has serious limitations since it does not identify the presence of many isolates that may be acquired during a patient's evolution owing to selection of bacterial flora associated with antibiotic pressure or other methods such as transmission by healthcare personnel [7] or as a result of recurrent infection [8].

It has already been pointed out that ESBL-producing organisms are emerging as a cause of infection, especially in outpatients; therefore, this study draws attention to the need to analyse in depth whether the criterion recommended by the CLSI is the most appropriate to study infections associated with ESBL-producing isolates since the criterion recommended by the EARSS identifies the presence of many isolates that are not identified by the former. These discrepancies may alter the data on the prevalence in a particular setting and should therefore be taken into account when designing local guidelines for empirical therapy and may have a great influence on the clinical utility of new tools that are being implemented to improve treatment of infections with these pathogens.

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