Sir,
In the last decade, *Actinobaculum schaalii*, a Gram-positive, facultative anaerobic, cocoid rod,1 has emerged as a uropathogen, mainly in elderly patients with underlying urological predispositions.2-4 Due to difficulties in cultivating *A. schaalii*, the incidence of the bacteria has probably been underestimated for many years.

Unless microscopy of Gram’s stains or wet smear is used for screening urinary samples, specimens will often not be cultured in an atmosphere supporting growth of *A. schaalii*. However, even when cultured in a CO2-enriched atmosphere *A. schaalii* can often only be identified by PCR, as a study has shown that in 90% of the cases it occurs together with fast-growing commonly known uropathogens, and thus is overlooked.3

*A. schaalii* is susceptible in vivo to nearly all β-lactams, such as amoxicillin or cephalosporins, but is resistant to trimethoprim.4 In a recent study of *A. schaalii*,4,5 the MICs of 12 different antimicrobial agents, including those commonly used for the treatment of urinary tract infections (UTIs), were determined. Pivmecillinam was not included, but it is often the antibiotic of choice for the oral treatment of UTIs in Scandinavia, e.g. in Denmark it accounts for approximately half of the defined daily doses used for UTIs.6,7

We therefore selected 18 clinical *A. schaalii* isolates and measured the MICs of mecillinam.

Fourteen of the isolates were obtained from urine specimens and four were from blood cultures. As a confirmatory test, all isolates were screened using a real-time PCR assay specific for *A. schaalii*, and the *A. schaalii* strain CCUG 27420 was used as a reference. The susceptibility testing was performed on Schaedler agar (Oxoid) supplemented with 5 μg/mL haemin, 1 μg/mL vitamin K1 and 5% sheep blood. An inoculum suspension adjusted to a turbidity equivalent to that of a 1 McFarland standard in 0.9% NaCl was spread on to the agar and incubated under anaerobic conditions at 35°C for 48 h. The MIC of mecillinam was determined for all isolates using Etest (AB bioMérieux, Solna, Sweden). *Bacteroides fragilis* ATCC 25285 was used as a quality control strain.

The susceptibility testing of mecillinam against the 18 *A. schaalii* isolates showed MIC values in the range 0.5–2.0 mg/L (Table 1), and the MIC90 were 1.0 and 2.0 mg/L, respectively. This is consistent with previous findings in which nine strains of *A. schaalii* were susceptible to mecillinam (aminocillin), with an MIC range of 0.25–1.5 mg/L, an MIC90 of 0.5 mg/L and an MIC90 of 1.0 mg/L.2

The present study suggests that pivmecillinam will be effective as an alternative to other β-lactams in the treatment of *A. schaalii* UTIs, although this β-lactam is not usually used against Gram-positive bacteria.6 The advantages of choosing pivmecillinam include the low level of resistance in urinary pathogens, in addition to the possibility of achieving very high urinary concentrations (>200 mg/L) due to active excretion into the urine.6

In conclusion, pivmecillinam as a first-choice antibiotic against UTIs probably covers *A. schaalii*, both when *A. schaalii* is identified in monocultures and more frequently when it occurs as a co-pathogen with common fast-growing uropathogens.

**Funding**
The study was carried out as part of our routine work.

**Transparency declarations**
None to declare.

**References**

**Table 1.** In vitro activity of mecillinam against 18 clinical isolates of *A. schaalii*

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>MIC (mg/L)</th>
<th>MIC90</th>
<th>MIC50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mecillinam</td>
<td>0.5–2.0</td>
<td>1.0</td>
<td>2.0</td>
</tr>
</tbody>
</table>

**Table 1.** In vitro activity of mecillinam against 18 clinical isolates of *A. schaalii*

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**Fluoroquinolone resistance in Escherichia coli and Klebsiella pneumoniae over 18 years: effect of different systems for eliminating duplicates**

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1Hospital de la Vega Baja, Orihuela, Alicante, Spain; 2Hospital General Universitario de Elche, Elche, Alicante, Spain; 3Universidad Miguel Hernández, Elche, Alicante, Spain
Sir,
The increase in resistance of Gram-negative bacilli is a serious public health problem and our aim was to determine the effect of different duplicate elimination criteria on the evolution of fluoroquinolone resistance in *Escherichia coli* and *Klebsiella pneumoniae* in our setting over a period of 18 years.

The criteria used are described in Figure 1. Antibiotic susceptibility was studied using microdilution with the Wider semiautomatic system (Soria Melguizo, Spain) and Etest (AB Biodisk, Solna, Sweden), following CLSI (formerly NCCLS) recommendations.

In *E. coli*, the mean percentage of fluoroquinolone-resistant strains according to the EARS-Net criterion was greater than that detected using the CLSI criterion, both when the total number of strains and when the number of extended-spectrum β-lactamase (ESBL)-producing strains was considered (28.4% versus 22.4% and 65.2% versus 60.5%, respectively). With regard to *K. pneumoniae*, we obtained 7.2% versus 6.3% and 52.2% versus 40.0%.

Analysis of the criterion of time showed that when the period considered for elimination of duplicates increased, the ciprofloxacin resistance rate also increased compared with the CLSI criterion.

When the EARS-Net criterion was applied, the prevalence of these microorganisms increased in both hospitalized and non-hospitalized patients, although this phenomenon was more important in the former than in the latter. All data are shown in Table 1.

The increase in fluoroquinolone resistance is a serious public health problem, especially in ESBL-producing strains, for which the therapeutic options are far more limited, and the local epidemiology of resistance has been considered a key tool in the control of this process. It is essential to apply a duplicate elimination criterion, since analysis of the data based on the total criterion does not reflect the real situation of resistance. What has not yet been definitively established is which criterion is the best to use in each case. The first isolate from each patient is an objective criterion, but has serious limitations since the same patient may frequently be infected by different strains of the same species during the course of their disease and hospital stay, or a strain may become resistant during treatment of the patient due to the selection of resistant mutants during antibiotic therapy. The EARS-Net criterion reflects more faithfully the real situation of resistance, but suggests that it is necessary to apply strict quality control in phenotypic microbiological studies to detect resistance.

Our study suggests that the new EARS-Net method of studying duplicates should be applied in order to detect changes in resistance patterns that may occur during the course of disease due to mutations of the microorganism causing the infection or superinfections by more resistant microorganisms.

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**Transparency declarations**
None to declare.

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**Keywords:** *E. coli*, *K. pneumoniae*, antimicrobial resistance, ciprofloxacin, epidemiology, antimicrobial susceptibility, extended-spectrum β-lactamases

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**Table 1.** Ciprofloxacin resistance in *E. coli* and *K. pneumoniae*: effect of different criteria for elimination of duplicates in samples from hospitalized and non-hospitalized patients

<table>
<thead>
<tr>
<th></th>
<th><em>E. coli</em></th>
<th></th>
<th><em>K. pneumoniae</em></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>total strains</td>
<td>inpatients</td>
<td>outpatients</td>
<td>total strains</td>
</tr>
<tr>
<td><strong>Criterion</strong></td>
<td>n</td>
<td>FQR (ESBL) (%)</td>
<td>n</td>
<td>FQR (ESBL) (%)</td>
</tr>
<tr>
<td>Total</td>
<td>19513</td>
<td>28.4</td>
<td>75.0</td>
<td>5282</td>
</tr>
<tr>
<td>CLSI</td>
<td>13552</td>
<td>22.4</td>
<td>60.5</td>
<td>3104</td>
</tr>
<tr>
<td>7 day</td>
<td>17574</td>
<td>28.5</td>
<td>68.9</td>
<td>4352</td>
</tr>
<tr>
<td>30 day</td>
<td>16902</td>
<td>28.2</td>
<td>68.8</td>
<td>4086</td>
</tr>
<tr>
<td>EARS-Net</td>
<td>16106</td>
<td>28.4</td>
<td>65.2</td>
<td>4182</td>
</tr>
</tbody>
</table>

n, number of strains; FQR, fluoroquinolone resistant.
Vitamin E supplementation in old mice induces antimicrobial activity and improves the efficacy of daptomycin in an animal model of wounds infected with methicillin-resistant Staphylococcus aureus

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Sir,

Antibiotic-resistant bacteria, such as methicillin-resistant Staphylococcus aureus (MRSA), are a recognized problem in healthcare settings, leading to refractory infections and potentially life-threatening illnesses.1 MRSA is often isolated from patient wounds in both the hospital and the community setting. A variety of factors determine whether a wound remains harmlessly colonized or succumbs to infection. Some of the most important factors include individual vulnerability to infection and the size and location of the wound, balanced against the number of microorganisms present and their virulence factors. Vulnerability factors include age, with the elderly being more prone to MRSA infection as they have a compromised immune response (immunosenescence), and underlying diseases, such as diabetes or cardiovascular disease, and malnutrition, all of which affect the wound-healing process.2,3

Daptomycin is a branched cyclic lipopeptide antibiotic of non-ribosomal origin and the prototype of the acidic lipopeptide family. It was approved in 2003 for the non-topical treatment of skin structure infections caused by Gram-positive pathogens, including MRSA, and in 2006 for the treatment of bacteremia.4,5 Vitamin E is a family of essential micronutrients composed of lipid-soluble tocopherols and tocotrienols with strong antioxidant and immunomodulating activity. Vitamin E supplementation is associated with increased resistance to several pathogens, especially in the elderly, who are at greater risk of inadequate dietary intake of vitamin E.6,7

We studied whether supplementation of old mice with vitamin E was effective in inducing antimicrobial activity and in increasing the effect of daptomycin in a mouse model of wound infection due to MRSA.

Sixteen-month-old male BALB/c mice (average weight 30 g) were assigned to four groups (n = 8 per group): a group pretreated with vitamin E alone (60 mg/kg by oral gavage 30 days prior to challenge); a group pretreated with vitamin E plus intraperitoneal daptomycin (7 mg/kg) after challenge; a group given only daptomycin (7 mg/kg) after challenge; and a control group that did not receive any treatment. Antibiotic was administered daily for 7 days. The experiments were repeated twice. Mice were anaesthetized by an intramuscular injection of ketamine and xylazine and hair on the back was shaved and the skin cleansed with 10% povidone–iodine solution. Using a 1.0 × 2.0 cm template, one full-thickness wound was established through the pannicus carnosus of the subcutaneous tissue on the back of each animal. A small gauze was placed over each wound and the gauze then inoculated with 5 × 10^7 cfu of S. aureus ATCC 43300. The pocket was closed by means of skin clips. This procedure resulted in a local abscess at 24 h. The procedure and facilities complied with ethical standards and followed the requirements of Commission Directive 86/609/EEC concerning the protection of animals used for experimental and other scientific purposes. Italian legislation is defined in D.L. No. 116 of 27 January 1992.

References


