other bacterial species and fungi. This has necessitated the use of laboratory media that contain various antibacterial and antifungal selective agents.1,2

Antibiotics have the tendency to select for resistant bacteria. For example, fluoroquinolones can select for mutants with gyrA mutations and/or that overexpress an efflux system.3 Therefore, there has been increasing concern that the use of antibiotics in Campylobacter isolation media may result in biased antibiotic susceptibility data sets.4 The antibiotics cefalothin, cefoperazone and rifampicin are incorporated in the currently used selective media. These agents are also substrates of efflux pumps, these media could select for mutants that overexpress the cmeABC, cmeDEF and/or other efflux systems in Campylobacter jejuni NCTC 11168. Alternatively, induction of these pump genes could allow Campylobacter spp. to survive the presence of antibiotics in media.

C. jejuni NCTC 11168 was cultured on standard Mueller–Hinton (MH) agar and also on three commonly used Campylobacter selective media (Table 1).

The plates were incubated under microaerophilic conditions for 48 h. Colonies were resuspended in MH broth, MH broth plus Blaser-Wang supplement, MH broth plus CCDA supplement and MH broth plus Preston supplement and incubated overnight. The suspensions were used as inocula for MIC determination and gene expression analysis for cmeABC and cmeDEF, as described previously.4,6 The antibiotics tested were ampicillin, cefalothin, chloramphenicol, ciprofloxacin, erythromycin, kanamycin, rifampicin, tetracycline and trimethoprim.

Susceptibility testing revealed no differences in the MIC of the tested antibiotics between C. jejuni NCTC 11168 cultured in standard media versus selective media. Comparison of the concentrations of the antibiotics used in these media and their MICs for C. jejuni NCTC 11168 showed that the concentrations used in the media were substantially below the MIC values (Table 1). Gene expression analysis also revealed no differences in the expression of cmeABC or cmeDEF between C. jejuni NCTC 11168 cultured in MH media compared with any of the three selective media. Therefore, it is concluded that selective culture media used to isolate Campylobacter spp. inhibit competition by other bacteria and fungi, but do not induce overexpression of known efflux pump genes, for which some of these agents are substrates. Therefore, any antibiotic resistance observed in isolates is unlikely to be an artefact due to media composition as has been suggested previously.2

### Acknowledgements

We are grateful to the Department of Environment, Food and Rural Affairs (formerly The Ministry for Agriculture, Fisheries and Food) for project grant CTA9903. L.J.V.P. is a recipient of the Bristol–Myers Squibb Non-restricted Grant in Infectious Diseases.

### References


### Table 1. Concentrations of agents in Campylobacter isolation media and MICs for C. jejuni NCTC 11168 (mg/L)

<table>
<thead>
<tr>
<th>Agent</th>
<th>Media</th>
<th>Concentration</th>
<th>MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphotericin B</td>
<td>Blaser-Wang (Oxoid SR0098); CCDA (Oxoid SR0155)</td>
<td>10</td>
<td>not done</td>
</tr>
<tr>
<td>Cycloheximide</td>
<td>Preston (Oxoid SR0117)</td>
<td>100</td>
<td>not done</td>
</tr>
<tr>
<td>Cefalothin</td>
<td>Blaser-Wang</td>
<td>14</td>
<td>32</td>
</tr>
<tr>
<td>Cefoperazone</td>
<td>CCDA</td>
<td>16</td>
<td>64</td>
</tr>
<tr>
<td>Polymyxin</td>
<td>Blaser-Wang; Preston</td>
<td>1.25</td>
<td>16</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>Preston</td>
<td>10</td>
<td>32</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>Blaser-Wang; Preston</td>
<td>5</td>
<td>128</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>Blaser-Wang</td>
<td>10</td>
<td>64</td>
</tr>
</tbody>
</table>

### Susceptibility testing Pasteurella multocida by BSAC standardized methodology


The BSAC Standardized Method Development Centre, Microbiology, City Hospital, Dudley Road, Birmingham B18 7QH, UK

Keywords: P. multocida, disc testing

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Nalidixic acid – – 30 27 28 – –
Ciprofloxacin 1 1 1 28 29 0.008 31–37
Cefotaxime 1 1 5 33 34 0.004 35–41
Penicillin 0.12 0.12 1 unit 21 22 0.12 24–28

NCTC 8489 were determined by disc testing 50 times on pre-
P. multocida However, acceptable limits for control strain
plates would be necessary for testing to avoid zone merging.
ampicillin and cefotaxime; this would mean that routinely two
were not determined because zone diameters were >_40 mm for
Acceptable limits for control strain
lin, ampicillin, cefotaxime, ciprofloxacin and tetracycline.

Methodology. In this study, we describe a disc susceptibility test-
more serious invasive infections. 1 Currently there are no rec-
lates was confirmed by API 20NE (bioMérieux, Basingstoke,
and disc susceptibility testing, Iso-Sensitest agar (Oxoid) sup-
cefin test (Oxoid, Basingstoke, UK). For MIC determinations
b
UK) and the presence of β-lactamase was detected by the nitro-
toxid, Basingstoke, UK) was used. An inocu-
llum of 10^4 cfu/spot and an inoculum equivalent to semi-conflu-
ance (an organism suspension equivalent to a 0.5 McFarland
standard diluted 1:100 in distilled water before inoculation) were
used for MICs and disc susceptibility testing, respectively. All
were incubated at 35–37°C for 18–20 h in an atmosphere
enriched with 4–6% CO₂. The antibiotics studied were penicil-
in, ampicillin, cefotaxime, ciprofloxacin and tetracycline. Acceptable limits for control strain P. multocida NCTC 8281
were not determined because zone diameters were ≥40 mm for
ampicillin and cefotaxime; this would mean that routinely two
plates would be necessary for testing to avoid zone merging.
However, acceptable limits for control strain P. multocida
NCTC 8489 were determined by disc testing 50 times on pre-
poured plates from Oxoid and bioMérieux and media poured in-
house to a depth of 3.5, 4 and 4.5 mm. Zone diameters from the
five media types were combined and 95 percentiles calculated.3
Applying the BSAC MIC breakpoints, susceptibility was deter-
moved.3
All of the organisms studied were β-lactamase negative. Mode MICs for each of the antibiotics tested were: penicillin,
0.06 mg/L (range 0.015–0.12 mg/L); ampicillin, 0.12 mg/L
(range 0.03–0.25 mg/L); cefotaxime, 0.004 mg/L (range 0.001–
0.004 mg/L); ciprofloxacin, 0.008 mg/L (range 0.004–
0.12 mg/L); and tetracycline, 0.25 mg/L (range 0.06–0.5 mg/L).
All of the isolates were susceptible to each of the antibiotics
tested based on comparison with the BSAC MIC breakpoints,
except for one isolate (Z1469) that had a ciprofloxacin MIC of
0.12 mg/L which was 16-fold higher than the mode MIC of
0.008 mg/L. Currently the BSAC recommends the use of a 30 μg
nalidixic acid disc for the detection of low-level fluoroquinolone
resistance in Gram-negatives.5 For Z1469 with reduced fluoro-
quinolone susceptibility, a zone of 14 mm was observed (a zone
of 26 mm with a ciprofloxacin 1 μg disc). For the control NCTC
8489 strain and for susceptible isolates, zones of ≥28 mm were
seen. It is therefore possible to use a 30 μg nalidixic acid disc to
detect reduced susceptibility to fluoroquinolones. The presump-
tive quinolone resistance-determining regions (QRDR) of gyrA
and parC for Z1469 and NCTC 8489 were amplified by PCR;
direct sequencing of purified PCR products was carried out by
automated fluorescent sequencing (MWG Biotech UK Ltd, Milton
Keynes, UK). No QRDR nucleotide sequence differences were
seen between Z1469 and NCTC 8489. Similar strains of
Haemophilus influenzae showing reduced quinolone suscepti-
bility, but lacking gyrA or parC mutations have been described.5
Recommendations for interpreting susceptibility and acceptable
limits for the control strain are given in Table 1.

## Acknowledgements

Centres providing clinical isolates: Derriford Hospital, Plym-
outh; William Harvey Hospital, Ashford; Crawley Hospital,
Crawley; Cheltenham Hospital, Cheltenham; Gloucester Royal
Hospital, Gloucester; Derbyshire Royal Infirmary, Derby; Mac-
clesfield District General Hospital, Macclesfield; University Hos-
ital of Wales, Cardiff; Queen Elizabeth 2 Hospital, Welwyn
Garden City; Leicester Royal Infirmary, Leicester; Blackpool
Victoria Hospital, Blackpool; Colchester General Hospital, Col-
chester; Bassetlaw District General Hospital, Worksop; Truro
Hospital, Truro; Royal Preston Hospital, Preston; City Hospital,
Birmingham.

## References

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In vitro activity of daptomycin against enterococci from nosocomial and community environments in Portugal

C. Novais, J. C. Sousa, T. M. Coque and L. V. Peixe on behalf of the Portuguese Resistance Study Group

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Keywords: cyclic lipopeptide antibiotics, clinical isolates, animal isolates, healthy volunteers, vancomycin-resistant enterococci

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†Members of the Portuguese Resistance Study Group are listed in the Acknowledgements

Table 1. Daptomycin activity against enterococci from different species and sources

<table>
<thead>
<tr>
<th>Species</th>
<th>Source</th>
<th>Isolates tested</th>
<th>Number of isolates at each daptomycin MIC (mg/L)</th>
<th>0.25</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>16</th>
<th>MIC(_{50})</th>
<th>MIC(_{90})</th>
</tr>
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<tbody>
<tr>
<td>E. faecalis</td>
<td>Hospital</td>
<td>VRE</td>
<td>32</td>
<td>3</td>
<td>11</td>
<td>16</td>
<td>2</td>
<td>2</td>
<td>2</td>
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<td></td>
<td></td>
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<tr>
<td></td>
<td>Hospital</td>
<td>VSE</td>
<td>99</td>
<td>3</td>
<td>8</td>
<td>52</td>
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<td>1</td>
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<tr>
<td></td>
<td>Sewage</td>
<td>VRE</td>
<td>3</td>
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<td>1</td>
<td>ND*</td>
<td>ND*</td>
<td>ND*</td>
<td>ND*</td>
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<tr>
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<td>VRE</td>
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<tr>
<td></td>
<td>River</td>
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<td>ND*</td>
<td>ND*</td>
<td>ND*</td>
<td>ND*</td>
<td>ND*</td>
<td>ND*</td>
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<tr>
<td></td>
<td>Healthy human</td>
<td>VRE</td>
<td>0</td>
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<td></td>
<td></td>
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<tr>
<td></td>
<td>Healthy human</td>
<td>VSE</td>
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<td>1</td>
<td>1</td>
<td>24</td>
<td>91</td>
<td>46</td>
<td>2</td>
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<tr>
<td></td>
<td>Poultry</td>
<td>VRE</td>
<td>11</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>1</td>
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<td></td>
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<tr>
<td></td>
<td>Swine</td>
<td>VRE</td>
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<td></td>
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<td>Swine</td>
<td>VSE</td>
<td>0</td>
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<tr>
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<td>102</td>
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<td>21</td>
<td>74</td>
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</tbody>
</table>

Sir,

Daptomycin is a novel cyclic lipopeptide antibiotic with a unique mechanism of action that involves disruption of multiple aspects of the plasma membrane function without penetration into the cytoplasm. This antibiotic exhibits rapid concentration-dependent killing and the occurrence of spontaneous resistant mutants is rare. Although several studies have shown good in vitro and in vivo activity of daptomycin against clinical isolates of enterococci,\(^1\)–\(^5\) its activity against animal and environmental enterococci has not been studied. The objective of this work was to investigate the activity of daptomycin against enterococcal isolates from nosocomial and community environments, including human faecal flora, animal food samples, sewage and rivers in Portugal, the European country currently with the highest prevalence of vancomycin-resistant enterococci (VRE) (Annual Report of European Antibiotic Resistance Surveillance System, EARSS, 2002; http://www.earss.rivm.nl). A high occurrence of VRE in the Portuguese community has also been shown by our group.\(^6\)

We studied 1151 isolates from: (i) patients at three hospitals in the centre and north of Portugal collected during 1996–2003 (\(n=251\)); (ii) human faecal samples from healthy volunteers living in the centre and north of the country and collected in 2001 (\(n=338\) isolates from 99 samples); (iii) swine faecal samples collected during 1997–1998 (\(n=17\) isolates from six samples); (iv) raw poultry products corresponding to 93 chicken lots and six turkey lots from 10 different brands and purchased at two different butcher shops—the samples were recovered during 1999–2001 (\(n=397\) isolates from 99 samples); (v) sewage water from Porto hospitals recovered in the period 2001–2002 (\(n=130\) isolates from 26 samples); (vi) river water samples from the Porto area in 2003 (\(n=18\) from six samples). A multiplex PCR assay was performed for species identification and detection of vancomycin resistance genes.\(^7\) Seven hundred and seventy-one isolates were resistant to three or more antibiotics (771/1151 isolates, 67%, data not shown). Thirty percent of the isolates (\(n=344/1151\)) were VRE of genotypes \(\text{vanA}\) (\(n=279/344, 81\%\)), \(\text{vanB}\) (\(n=4/344, 1\%\)) or \(\text{vanC1}\) (\(n=61/344, 18\%\)). Twenty-two percent (\(n=250/1151\)) showed high-level resistance (HLR) to...