Evaluation of media available for testing the susceptibility of
Pseudomonas aeruginosa by BSAC methodology

Jennifer Andrews¹*, Rebecca Walker¹ and Anna King² for the BSAC Working Party on
Sensitivity Testing

¹Department of Microbiology, City Hospital NHS Trust, Birmingham, UK and ²Department of Microbiology,
St Thomas’ Hospital, London, UK

BSAC methodology was used to study media available from four manufacturers for suscept-
ibility testing of Pseudomonas aeruginosa in the UK and Ireland. Fifty isolates of P. aeruginosa,
including ATCC and NCTC control strains, were studied by disc testing methods by 20 centres
around the UK and Ireland and by MIC determination at the BSAC Standardized Method Develop-
ment Centre (SMDC) in Birmingham, UK. Although many of the antibiotics tested gave similar
zone diameters for a particular strain–antibiotic combination irrespective of the medium used
for testing, significant differences in media performance were noted for the aminoglycosides,
imipenem and colistin. The data generated showed that for testing aminoglycosides, zone dia-
 meters on two of the agars were significantly larger than those on Oxoid IsoSensitest agar—the
recommended medium for the BSAC method. For imipenem, zones were smaller on one of the
agars and for colistin, zones were larger on one of the agars in comparison with those on Oxoid
agar. Scattergram analysis of zone diameters and MICs of colistin indicated that the zone di-
meter breakpoints should be adjusted. Media purported to be equivalent to Oxoid IsoSensitest
agar do not give the same zone sizes with some agents and should not be used until standards
for media performance have been established and the media have been shown to perform
adequately.

Introduction

For a particular organism, antimicrobial agent and disc
content, many variables have been shown to affect the size
of zones of inhibition, but, amongst the most critical is the
culture medium.¹ From early experiences with the National
Committee for Clinical Laboratory Standards (NCCLS)
method, it was clear that different batches of Mueller–Hinton
agar affected the interpretation of susceptibility, and that
Pseudomonas aeruginosa tested against aminoglycoside
antibiotics was most affected.² These observations led to
the NCCLS devising protocols for evaluating dehydrated
Mueller–Hinton agar to control media performance.³,⁴

In 1998 the British Society for Antimicrobial Chemo-
therapy (BSAC) published a standardized method of disc sus-
cceptibility testing.⁵ The medium stipulated was IsoSensitest
agar (ISA) (Oxoid, Basingstoke, UK) or one shown to be of
‘equal performance’. The aim of this study was to compare
the performance of media currently marketed with claims to
meet this requirement. Testing the susceptibility of P. aeru-
ginosa was chosen because it has been shown to be a sensitive
indicator of variation in medium composition.⁶,⁷

Material and methods

Organisms studied

Fifty P. aeruginosa, comprising 48 clinical isolates and two
control strains, NCTC 10662 and ATCC 27853, were studied.
Included in this collection were 18 isolates that were used in a
field study during the development of the BSAC Standardized
Disc Testing Method.⁸ The two control strains were provided
to participants separately so that they were tested as both
coded samples and known controls. Identification of the

*Corresponding author. Tel: +44-121-507-5693; Fax : +44-121-551-7763; E-mail: jenny.andrews@cityhospbham.wmids.nhs.uk

© 2002 The British Society for Antimicrobial Chemotherapy
isolates was confirmed by oxidase test and with API 20NE identification kits (bioMérieux, Basingstoke, UK). The presence and identification of mechanisms of resistance to aminoglycoside antibiotics were inferred from resistance patterns obtained after determining MICs of amikacin, apramycin, gentamicin, isepamicin, kanamycin, neomycin, netilmicin and tobramycin and comparative zone diameters for epi-sisomicin, fortimicin, 2' netilmicin and 6' netilmicin.9

**MICs**

MICs were determined with methodology based on that recommended by the BSAC.5 Media used were ISA (Oxoid), Isotonic Sensitivity Test agar (Mast Diagnostics, Merseyside, UK) (Mast), Balanced Sensitivity Test medium (Becton Dickinson, Oxford, UK) (Difco) and LAB M Susceptibility Test ‘ISO’ agar (International Diagnostics Group plc, Bury, UK) (LAB M), and were prepared following the manufacturers’ instructions. Standard antimicrobial powders were obtained from the following sources: ciprofloxacin, Bayer AG (Wuppertal, Germany); gentamicin and ofloxacin, Aventis (Paris, France); amikacin and aztreonam, Bristol Myers Squibb (Princeton, NJ, USA); ceftazidime, ticarcillin and clavulanic acid, Glaxo SmithKline (Stevenage, UK); imipenem, Merck Sharpe & Dohme Ltd (Hoddesdon, UK); colistin sulphate, Pharmax (Bexley, UK); netilmicin, Schering Plough (Kenilworth, NJ, USA); piperacillin and tazobactam, Wyeth Laboratories (Pearl River, NY, USA); tobramycin, Lilly Laboratories (Basingstoke, UK); meropenem, AstraZeneca Pharma (Macclesfield, UK). All antimicrobials were prepared from 10000 mg/L frozen stock solutions (−70°C), with the exception of gentamicin, which was stored at 4°C, and tazobactam and colistin, which were prepared on the day of MIC testing. Fixed concentrations of 4 mg/L tazobactam and 2 mg/L clavulanic acid were combined with piperacillin and ticarcillin, respectively. Doubling dilution concentrations based on 1 mg/L were used for all antibiotics. In addition, for the aminoglycosides tested against the two control strains, a range increasing by 0.1 mg/L incremental steps was tested to detect small differences in MIC. MIC determinations on all four media were undertaken by the SMDC.

**Disc diffusion susceptibility testing**

Twenty diagnostic laboratories, from throughout the UK and Ireland, participated in the disc diffusion study (see Acknowledgements). The laboratories were divided into four groups of five to achieve an even geographical distribution within each group. Each group used one of the four media and each centre was coded by medium supplier as follows: Oxoid: OA, OB, OC, OD, OE, etc.

Disc diffusion testing was undertaken following BSAC recommendations,10 and participating laboratories were provided with written instructions. The same media and antibiotics were used as those for MIC determinations. Participants prepared the media following the manufacturers’ instructions, and were asked to submit details to the BSAC Standardized Method Development Centre (SMDC) of how they had prepared and stored agar plates for use in the trial. Antibiotic discs were supplied by Oxoid, in sufficient quantities for all participating laboratories to use the same batch numbers.

**Analysis of data**

The data were analysed by one-way analysis of variance and the Mann–Whitney test for zone diameters and MIC values respectively, and differences in rates of susceptible and resistant interpretation depending on medium used for testing were examined by the χ² test (Statview, Cary, NC, USA). MIC values for control strain *P. aeruginosa* NCTC 10662 were compared, where possible, with values published by the BSAC11 and zone diameters for both control strains were compared with BSAC reference ranges.10 Individual zone diameters for the test isolates were compared with the interpretative values given in the BSAC recommendations.10 In addition, the rates of resistant and intermediate interpretation were compared for each medium and medium–laboratory combination where applicable.

**Results**

Colonial appearance was similar on Oxoid and Difco media, with colonies being larger than those on Mast or LAB M media (observed by SMDC).

**MICs on different media**

**β-lactam antibiotics.** MICs of all β-lactam antibiotics tested, except imipenem, for the control strains on each of the media tested were within one two-fold concentration of the published BSAC target values. No differences in MIC distribution or interpretation of susceptibility were observed between any of the media (*P > 0.6*) (data not shown). With imipenem, all MICs for the control strains were within one two-fold dilution of published BSAC target values, except for the NCTC 10662 control tested on Difco medium, where the MIC was 8 mg/L, which was eight-fold higher than the published value. MICs tended to be lower on Oxoid medium and the differences were significant when results on Oxoid medium were compared with those on Difco and LAB M media (*P ≤ 0.01*). Differences in MIC did not affect the interpretation of susceptibility, which were the same on all media except for the control strain mentioned above.

**Ciprofloxacin and ofloxacin.** Ofloxacin MICs for the control strains were within one two-fold concentration of the
published value on all four media. There were no significant differences in MIC distribution or interpretation of susceptibility for test strains between the four media studied (data not shown). Results for ciprofloxacin were similar to those obtained for ofloxacin except that MICs on Oxoid medium were significantly higher than on Mast medium ($P = 0.02$).

**Colistin.** There is no BSAC MIC target value published for *P. aeruginosa* ATCC 27853. However, on the four media, MICs ranged from 0.5 to 1 mg/L. For the *P. aeruginosa* NCTC 10662 control strain, colistin MICs were within one two-fold dilution of target values on Oxoid and Difco media, but were four-fold lower than the published value of 2 mg/L on Mast and LAB M media. MICs for the 50 test organisms were generally similar on Oxoid and Difco media and were one two-fold dilution higher when compared with values obtained on the other two media. Differences in MIC did not affect the interpretation of susceptibility, with all isolates susceptible to colistin irrespective of the medium used (data not shown).

**Aminoglycosides.** Results for the two control strains, *P. aeruginosa* NCTC 10662 and ATCC 27853, are shown in Table 1. All MICs of the aminoglycosides for NCTC 10662 were within the acceptable two-fold concentration range, except for the tobramycin MIC on Mast medium which was four-fold lower than the published value.

MICs of gentamicin, tobramycin and amikacin for ATCC 27853 were within the acceptable two-fold concentration range on Difco and LAB M media but were two-fold lower than the published value for tobramycin on Oxoid medium and for all three aminoglycosides on Mast medium. There is no published value for netilmicin for this control strain but results were lower on Mast and LAB M media than on Oxoid and Difco.

MICs of aminoglycosides for the 50 isolates were in agreement between Oxoid and Difco media ($P \geq 0.2$) and between Mast and LAB M media ($P \geq 0.5$). MICs on Oxoid and Difco media were generally two-fold higher than those on Mast and LAB M media. The resistance mechanism (Table 2) was used as the definitive criterion for categorizing the 50 isolates as susceptible, intermediate or resistant. Results obtained on the four media are shown in Figure 1. For the isolates categorized as susceptible, gentamicin, tobramycin and amikacin MICs were generally correct on all media. However, netilmicin MICs were higher than expected, and were interpreted as indicating intermediate susceptibility for nine and 14 of 35 isolates on Oxoid and Difco media, respectively.

For the five strains with reduced susceptibility attributed to reduced permeability to aminoglycosides, all were within the susceptible range for tobramycin on all the media, and for the other aminoglycosides the results showed considerable variability (Figure 1). For the isolates categorized as resistant to

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>BSAC target MIC (mg/L)</th>
<th>Oxoid</th>
<th>Difco</th>
<th>Mast</th>
<th>LAB M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control strain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCTC 10662</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gentamicin</td>
<td>1</td>
<td>1</td>
<td>1.1 (2)</td>
<td>1 (1)</td>
<td>0.5 (0.5)</td>
</tr>
<tr>
<td>amikacin</td>
<td>2</td>
<td>1.9 (2)</td>
<td>1.1 (2)</td>
<td>0.6 (1)</td>
<td>0.7 (1)</td>
</tr>
<tr>
<td>netilmicin</td>
<td>0.5</td>
<td>2</td>
<td>0.2 (0.25)</td>
<td>0.8 (1)</td>
<td>0.1 (0.12)</td>
</tr>
<tr>
<td>tobramycin</td>
<td>0.5</td>
<td>0.8 (1)</td>
<td>0.6 (1)</td>
<td>0.7 (1)</td>
<td>0.1 (0.12)</td>
</tr>
<tr>
<td>ATCC 27853</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gentamicin</td>
<td>1</td>
<td>1.2 (2)</td>
<td>1.1 (2)</td>
<td>0.6 (1)</td>
<td>0.7 (1)</td>
</tr>
<tr>
<td>amikacin</td>
<td>2</td>
<td>0.7 (1)</td>
<td>0.6 (1)</td>
<td>0.7 (1)</td>
<td>0.1 (0.12)</td>
</tr>
<tr>
<td>tobramycin</td>
<td>0.5</td>
<td>0.8 (1)</td>
<td>0.6 (1)</td>
<td>0.7 (1)</td>
<td>0.1 (0.12)</td>
</tr>
</tbody>
</table>

Table 1. MICs (0.1 mg/L incremental steps) of the aminoglycosides, gentamicin, amikacin, netilmicin and tobramycin for the *P. aeruginosa* control strain NCTC 10662 tested on four different media, compared with BSAC published target values.
the aminoglycosides by virtue of possessing modifying enzymes (Table 2), there was >90% agreement between MIC values and the resistance profile for gentamicin and tobramycin. Agreement was less for the 10 netilmicin-resistant strains (30% on Mast and LAB M and ≥70% on Difco and Oxoid media) and the three amikacin-resistant isolates were not detected on any of the media (Figure 1).

Disc diffusion testing on different media

*P. aeruginosa* control strains NCTC 10662 and ATCC 27823. Combined data for the four media, when controls were tested once as a known control and once as a coded sample, are shown in Table 3 (for the discs where BSAC acceptable limits were available for comparison). Most observations outside the limits of acceptability for both controls were seen when testing gentamicin and amikacin on Mast medium, where zones were larger than the upper limit of acceptability, and when testing imipenem on LAB M medium, on which, 60–100% of zones for the ATCC and NCTC controls were smaller (observed range for NCTC and ATCC control of 15–19 mm and 17–27 mm, respectively) than the acceptable ranges (acceptable range for NCTC, 20–27 mm; ATCC control, 23–28 mm).

Differences between centres using the same medium for testing

Results for one isolate were not included in the analysis because some centres observed two colony variants.

Zone diameter data for each of the media submitted by the testing laboratories were compared to see if there were any statistically significant differences between zones of inhibition. The number of occasions where a significant difference was observed was recorded and the totals for each of the medium–antibiotic combinations obtained. Irrespective of medium used, most differences between laboratories were observed when testing amikacin and colistin; most differences between laboratories using the same medium for testing were seen with Difco medium; no differences were observed when testing piperacillin alone and in combination with tazobactam, ticarcillin, gentamicin or tobramycin (data not shown).

Interpretation of susceptibility

MIC data obtained by the SMDC, on the same medium as used for disc testing, was used to interpret the susceptibility of the 49 isolates, except in the case of the aminoglycosides, where the presumed mechanism of resistance was used to categorize susceptibility. Agreement between interpretation of susceptibility by MIC and disc diffusion methods was observed for piperacillin and ticarcillin (alone and in com-

<table>
<thead>
<tr>
<th>Number of strains</th>
<th>Gentamicin-sensitive</th>
<th>Netilmicin-sensitive</th>
<th>Amikacin-sensitive</th>
<th>Apramycin-sensitive</th>
<th>Isepamicin-sensitive</th>
<th>Fortimicin-sensitive</th>
<th>Epi-sisomicin-sensitive</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>33</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>7</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>3</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>1</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
</tr>
</tbody>
</table>

Table 2. Presence and identification of mechanisms of resistance to aminoglycosides for the 50 isolates of *P. aeruginosa*, based on susceptibility patterns to an extended range of aminoglycosides.
Culture media for susceptibility testing

Figure 1. Comparison of interpretation of susceptibility by MIC testing with predicted mechanism of resistance to aminoglycosides on four media used for testing *P. aeruginosa*. *Number of isolates predicted to be susceptible (S), intermediate (I) or resistant (R) on the basis of the inferred resistance pattern.

Table 3. Performance of susceptibility testing of control strains *P. aeruginosa* NCTC 10662 and ATCC 27823 judged by combining zone diameter data for five centres (each strain tested once as a known control and once as a coded sample) on four different media.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Disc content (µg)</th>
<th>Oxoid</th>
<th>Mast</th>
<th>Difco</th>
<th>LAB M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceftazidime</td>
<td>30</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>75</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Piperacillin–tazobactam</td>
<td>75 + 10</td>
<td>9/10</td>
<td>9/10</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Imipenem</td>
<td>10</td>
<td>9/10</td>
<td>✓</td>
<td>✓</td>
<td>0/10</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>1</td>
<td>✓</td>
<td>6/10</td>
<td>8/10</td>
<td>✓</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>10</td>
<td>✓</td>
<td>3/10</td>
<td>8/10</td>
<td>6/10</td>
</tr>
<tr>
<td>Amikacin</td>
<td>30</td>
<td>✓</td>
<td>4/10</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>

For imipenem, there was general agreement in interpretation of susceptibility between MIC and disc testing when Oxoid, Mast and Difco media were used for testing (data not shown). However, in the case of LAB M medium, 22% of organisms were interpreted as resistant to imipenem by MIC testing, compared with 28–38% resistant by disc testing, by the five centres using this medium. For meropenem, no differences between MIC and disc interpretation were observed with any of the media used for testing.

The resistance profile was used to categorize the isolates as susceptible, intermediate or resistant for the aminoglycosides. A summary of interpretation by disc diffusion testing compared with the resistance profile is shown in Figure 3. For the susceptible isolates there was >88% agreement between...
the resistance profile and results of disc diffusion testing for the four aminoglycosides on all the media. For the five isolates with reduced permeability to the aminoglycosides >50% of the 25 zone diameters indicated reduced susceptibility to gentamicin and netilmicin on Oxoid and Difco media, but on the other two media only 4–12.5% of observations indicated reduced susceptibility. All isolates were interpreted as susceptible to tobramycin on all the media and all were considered susceptible to amikacin except on Difco medium, where 37.5% of the observations indicated reduced susceptibility. For the resistant isolates >90% of disc results agreed with the resistance mechanism for gentamicin and tobramycin on all the media. Agreement between resistance mechanism and disc results for netilmicin varied from 28.6% to 76% depending on the medium and the majority of amikacin results for the resistant isolates were incorrectly interpreted as susceptible.

Discussion
Susceptibility testing is an essential aid to ensuring appropriate antimicrobial chemotherapy, and provides surveillance data. Both require that the testing method is robust and reli-
able. Disc diffusion methods should therefore give equivalent zones irrespective of the laboratory performing the test. Reproducibility is based on consistency of technical details including the medium used. The BSAC method stipulates ISA, or medium shown to have the same performance. Several other media are claimed to have the same performance as ISA and in this study we compared the performance of three other media with that of ISA for testing the susceptibility of *P. aeruginosa*.

Although many of the antibiotics tested were unaffected by the media, there were some significant differences for aminoglycosides, colistin and imipenem. All of the strains were sensitive to colistin by MIC tests, irrespective of media used for testing. However, 6.1–64.5% were resistant by disc test depending on the medium used. This observation is not surprising, given that colistin is a large molecule and its diffusion may be more susceptible to medium composition. As all of these strains were susceptible to colistin by MIC, and because resistance to colistin in *P. aeruginosa* is rare, it is suggested that the zone diameter breakpoint for colistin be reviewed by the BSAC Working Party on Sensitivity Testing. These data for aztreonam have also shown that the current zone diameter breakpoint for aztreonam (34 mm = sensitive) is inappropriate, and a revised zone diameter of 23 mm is suggested.

More difficult to explain are the differences observed with imipenem for both MIC and disc testing. This was the only β-lactam antibiotic that showed differences in MIC values depending on medium used for testing. Imipenem is known to be unstable and is affected by reducing substances present in media (John Hobson, Mast Diagnostics, personal communication), which may explain these differences. This instability is not shared by the other carbapenem tested, meropenem, with which there were no significant differences between MIC values and between zone diameters on any of the media.

This study confirmed that susceptibility testing of *P. aeruginosa* to the aminoglycosides is problematic. It has also been shown that medium composition has a major effect on the activity of aminoglycosides and this has led to publication of an approved standard for dehydrated media by the NCCLS. In this study, analysis of aminoglycoside susceptibility was based on inferring the resistance mechanisms of all the strains studied. These data show that the MIC breakpoints for gentamicin suggested by the Working Party in 1991 are still relevant for clinical isolates, and that the tentative amendment made to the susceptible MIC breakpoint (raised from 1 to 2 mg/L) based on MIC values for a control strain, is not appropriate, because strains with reduced permeability would be considered susceptible. For this reason the 1991 breakpoints have been used for analysis of results in this study. Mast and LAB M media did not reliably detect strains with reduced susceptibility to gentamicin by disc testing. These data show that tobramycin is less affected by medium composition than the other aminoglycosides tested.

The strains with reduced permeability to aminoglycosides were not differentiated from the susceptible population for tobramycin. MICs of tobramycin for reduced-permeability isolates are often 1 mg/L, so these are susceptible to this drug. The three isolates with aminoglycoside-modifying enzymes affecting amikacin were not considered resistant by the BSAC disc method and, although the amikacin MICs for these strains were close to the breakpoint, these results were unexpected.

Although cationic concentration may play a part in the difference in performance between the media observed for the activity of the aminoglycosides and possibly colistin and ciprofloxacin, it is also possible that differing nutritional content may contribute to differences in the sizes of zones of inhibition. Growth was less luxuriant on Mast and LAB M media, resulting in larger zones of inhibition, particularly with the organisms isolated from patients with cystic fibrosis, where zones of inhibition, around gentamicin discs, on Mast medium often exceeded 40 mm, compared with zones of 24 mm on Difco and Oxoid media.

Examination of the procedures used by individual laboratories for the preparation and storage of plates showed no apparent difference to explain the differences in zone diameter observations.

Although it is possible to move the zone diameter breakpoints of an antibiotic to improve interpretation of susceptibility, if zone diameters are to be used to monitor subtle changes in susceptibility or to monitor test performance, then it is important that media used for testing give equivalent zone diameter measurements. It is clear from these data that other media tested were not the same as Oxoid IsoSensitest and should not be used for the BSAC method at this time. The BSAC needs to devise a standard for the performance of dehydrated media, particularly with regard to the activity of the aminoglycosides, colistin and imipenem. In common with other organizations who give recommendations for susceptibility testing, it may be necessary to have a large archive sample of the approved medium with which future batches are compared to ensure equivalent performance. Whether performance of media will be judged by MIC determinations, acceptable zone diameters for control strains or a combination of both will have to be decided. This study has also shown the value of using clinical isolates with known mechanisms of resistance to monitor performance.

**Acknowledgements**

We would like to thank the following Departments of Microbiology for taking part in this study: Rotherham District General Hospital; Hammersmith Hospital, London; PHLS, Derriford Hospital, Plymouth; PHLS, Bristol Royal Infirmary; University Hospital Birmingham NHS Trust; Manchester...
Royal Infirmary; St Thomas’ Hospital, London; Royal Gwent Hospital, Newport; Freeman Hospital, Newcastle-upon-Tyne; Royal Hallamshire Hospital, Sheffield; Great Ormond Street Hospital for Sick Children, London; Royal Hampshire County Hospital, Winchester; New Cross Hospital, Wolverhampton; Southmead Hospital, Bristol; Leighton Hospital, Crewe; Ealing Hospital, Southall; Mount Carmel Hospital, Dublin; PHLS, Addenbrookes Hospital, Cambridge; PHLS, Truro, Cornwall. We would also like to thank Dr K. Shannon for assistance with graphical presentation of the data.

References


