Impact of changed co-amoxiclav susceptibility testing formats on apparent resistance rates for bloodstream *Escherichia coli* in a long-term surveillance

Rosy REYNOLDS[1], Shazad MUSHTAQ[2], Michael K ALLEN[3], Carolyne HORNER[4,a], Christopher LONGSHAW[5], and David M LIVERMORE[6*]

[1]Bristol Medical School, University of Bristol, Bristol, UK; [2]UK Health Security Agency, London; [3]Merck Sharp & Dohme (UK) Limited, London, UK; [4]BSAC, Birmingham, UK; [5]Shionogi B.V., London, UK and [6]University of East Anglia, Norwich, UK

[a] Present address. Una Health Ltd, Stoke-on-Trent, UK

* corresponding author

David M Livermore
Professor of Medical Microbiology
Floor 2, Bob Champion Research & Educational Building,
James Watson Road,
University of East Anglia,
Norwich Research Park,
NORWICH, NR4 7UQ

Tel +44-(0)1603-597-568
d.livermore@uea.ac.uk

SIR, Until 2014 the BSAC advocated that co-amoxiclav MICs should be determined using a 2:1 gravimetric ratio of amoxicillin and clavulanate. Subsequently, following adoption of EUCAST breakpoints, this advice changed to using a fixed 2 mg/L of clavulanate. The effect was to lower the breakpoint for Enterobacterales from systemic infections from 8+4 to 8+2 mg/L. We report on the consequences for resistance prevalence among *Escherichia coli* as recorded in the BSAC Bacteraemia Antimicrobial Resistance Surveillance Programme.

This programme ran from 2001 to 2019 and has been extensively described.[1] Succinctly, microbiology laboratories across the United Kingdom and Ireland sent consecutive isolates, according to a per-species quota, to PHE's Antimicrobial Resistance and Healthcare Associated Infections (AMRHAI) Reference Unit for centralised testing. The number of laboratories participating annually was 24-25 from 2001-09 and again from 2016-19 but increased to 38-40 from 2010 to 2015. The programme sought a total of 250 *E. coli* isolates from these sites until 2007 and 500 thereafter; actual numbers collected ranged from 242 to 250 (mean, 247) annually in the 2001-07 period and 467 to 548 (mean, 508) subsequently.

Species identification used colorimetric agars (CHROMagar™ Orientation, CHROMagar, Paris, France), with API20E strips (bioMérieux, Basingstoke, UK) for any confirmatory tests until 2011 and MALDI-ToF (Bruker Daltonics, Bremen, Germany) thereafter. Susceptibility testing was by BSAC agar dilution on IsoSensitest media (Oxoid/Thermofisher, Basingstoke, UK), with a 2:1 amoxicillin:clavulanate ratio until 2013 and a fixed 2 mg/L clavulanate subsequently. Parallel tests with both methods were run in 2001 and 2002.

The proportion of *E. coli* isolates found susceptible to co-amoxiclav by year is illustrated in panel A of the figure. In 2001-2, when both formats were tested, resistance rates with the fixed 2 mg/L clavulanate were 13.3 percentage points (95% CI 9.9 to 16.8, paired data) above those with the 2:1 ratio. Thereafter, from 2003, there were no convincing trends towards more or less resistance during periods when the test format remained constant. However, following the switch to the fixed 2 mg/L format in 2014, with its reduction in the effective breakpoint from 8+4 to 8+2 mg/L, recorded resistance rose by 10.8 percentage points

(95% CI 7.7 to 13.8; unpaired data, adjusted for centre clustering with robust standard errors, possibly confounded by yearly effects). The resistance rate was under 30% in 7 of the 11 years (2003–2013) when only the 2:1 ratio was tested but exceeded 39% in 5 of the 6 following years, when testing was only with the fixed 2 mg/L clavulanate.

If only amoxicillin-resistant *E. coli* isolates were considered, as those where clavulanate might be expected to have an effect, the resistance rate to co-amoxiclav was under 50% in 7 of 11 years when only the 2:1 ratio was tested but exceeded 60% in 5 of the 6 later years, when testing was performed with a fixed 2 mg/L clavulanate (fig. panel B).

The present results are unsurprising, given the higher inhibitor concentration at breakpoint with the 2:1 ratio. They are in keeping with data published by others who tested both formats in parallel,[2,3,4] but extend knowledge by showing the consequences for a long-term surveillance. They are of practical importance because co-amoxiclav is one of the mostly widely used intravenous antibiotics across Europe, because *E. coli* accounts for 30-33% of all bacteraemias,[5] and because similar changes in testing modality and contingent breakpoints apply in many other countries adopting EUCAST methodology.

There remains the issue of which testing conditions better represent the patient. This was explored by Delgardo-Valverde *et al.*,[4] who found that clinical response correlated better with use of a fixed 2 mg/L of inhibitor, though poor outcomes were associated with MICs above 16+2 rather than above the 8+2 mg/L breakpoint. Caveats are that the analyses are complicated by: (i) inclusion of other species besides *E. coli,* some of them with inherent co-amoxiclav resistance, and (ii) analysis of ratio testing largely in relation to the 16+8 mg/L CLSI breakpoint, which has a clavulanate concentration sufficient to inhibit the growth of some *E. coli*, rather than in relation to the previous EUCAST 8+4 mg/L value.

Given these uncertainties, and the high rates of resistance evident here and in PHE data for bloodstream *E.* coli,[5] we would discourage empirical use of co-amoxiclav in severely-ill patients where this pathogen is likely.

Acknowledgements.

We are grateful to all sites that have contributed isolates, data, and time to the BSAC Antimicrobial Resistance Surveillance Programme over almost 20 years. RR acknowledges grant support from the BSAC to finance analysis of accumulated data from its surveillance programmes, also support from the NIHR Health Protection Research Unit in Behavioural Science and Evaluation at University of Bristol.

Funding

The BSAC Resistance Surveillance Programme was wholly supported by the pharmaceutical industry. A list of companies that provided sponsorship throughout the surveillance programme is available at http://www.bsacsurv.org.

Transparency Declaration

MA is a Trustee of the BSAC Council and is employed by Merck Sharp & Dohme (UK) Limited, London, UK, CL: is a Trustee of the BSAC Council and is employed by Shionogi B.V., DML: Advisory Boards or ad hoc consultancy Accelerate, Antabio, Centauri, Entasis, Integra-Holdings, Meiji, Menarini, Mutabilis, Nordic, Paion, ParaPharm, Pfizer, QPEX, Russian Direct Investment Fund, Shionogi, Summit, T.A.Z., VenatoRx, Wockhardt, Zambon, Paid lectures – bioMérieux, Beckman Coulter, Cardiome, GSK, Hikma, Merck/MSD, Menarini, Nordic, Pfizer, and Shionogi. Relevant shareholdings or options – Dechra, GSK, Merck, Perkin Elmer and Pfizer, amounting to less than 10% of portfolio value. He also has nominated holdings in Arecor, Avacta, C4X Discovery, Creo Medical, Diaceutics, Evgen, Faron, Genedrive, Maxcyte, Poolbeg, Renalytics AI, Synairgen and Trellus (all with research/products pertinent to medicines or diagnostics) through Enterprise Investment Schemes but has no authority to trade these shares directly. SM is a member of PHE's Antimicrobial Resistance and Healthcare Associated Infections Reference Unit, which has received financial support for conference attendance, lectures, research projects, or contracted evaluations from numerous sources, including Accelerate Diagnostics, Achaogen Inc., Allecra Therapeutics, Amplex,

AstraZeneca UK Ltd, AusDiagnostics, Basilea Pharmaceutica, Becton Dickinson Diagnostics, bioMérieux, Bio-Rad Laboratories, BSAC, Cepheid, Check-Points B.V., Cubist Pharmaceuticals, Department of Health, Enigma Diagnostics, Food Standards Agency, GlaxoSmithKline Services Ltd, Helperby Therapeutics, Henry Stewart Talks, IHMA Ltd, Innovate UK, Integra holdings, Kalidex Pharmaceuticals, Melinta Therapeutics, Merck Sharpe & Dohme Corp, Meiji Seika Pharma Co. Ltd, Mobidiag, Momentum Biosciences Ltd, Neem Biotech, Nordic Pharma Ltd, Norgine Pharmaceuticals, Paratek Pharmaceuticals, Rempex Pharmaceuticals Ltd, Roche, Rokitan Ltd, Smith & Nephew UK Ltd, Shionogi & Co. Ltd, Trius Therapeutics, T.A.Z., VenatoRx Pharmaceuticals and Wockhardt Ltd. CH and RR have nothing to declare.

References

1) Reynolds R, Hope R, Williams L *et al.* Survey, laboratory and statistical methods for the BSAC Resistance Surveillance Programmes. *J Antimicrob Chemother* 2008; **62 Suppl 2:** ii15–ii28

2) Diez-Aguilar M, Morosini MI, Lopez-Cerero L *et al.* Performance of EUCAST and CLSI approaches for co-amoxiclav susceptibility testing conditions for clinical categorization of a collection of Escherichia coli isolates with characterized resistance phenotypes. *J Antimicrob Chemother* 2015; **70:** 2306–10.

3) Leverstein-van Hall MA, Waar K, Muilwijk J *et al.* Consequences of switching from a fixed 2: 1 ratio of amoxicillin/clavulanate (CLSI) to a fixed concentration of clavulanate (EUCAST) for susceptibility testing of *Escherichia coli. J Antimicrob Chemother* 2013; **68:** 2636–40.

4) Delgado-Valverde M, Valiente-Mendez A, Torres E *et al.* MIC of amoxicillin/clavulanate according to CLSI and EUCAST: discrepancies and clinical impact in patients with bloodstream infections due to Enterobacteriaceae. *J Antimicrob Chemother* 2017; **72:** 1478–87.

5) Public Health England. English Surveillance Programme for Antimicrobial Utilisation and Resistance (ESPAUR). Report for 2019-2020. Available via https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/936199/ESPAUR_Report_2019-20.pdf

Figure. Resistance trends to co-amoxiclav, according to testing format. Panel A, all *E. coli* isolates collected; Panel B, amoxicillin-resistant (MIC >8 mg/L) isolates only.