

**BSAC User Group Meeting
Royal College of Physicians, London
Friday 8 June 2007**

Shift to European (EUCAST) breakpoints - the impact on the BSAC recommendations *Professor Alasdair MacGowan, Bristol*

Pancreatitis and prostatitis

Q: Most antibiotics don't penetrate very well into pancreatic and prostatic tissues. In a clinical situation you haven't got a clue as to the micro-organism causing the pancreatitis or prostatitis. Would it be better if the BSAC concentrated on tissue penetration of inflamed organs and tried to determine the best antibiotic or best combinations?

A: The problems really lie in two areas, the methodologies we use to determine tissue concentrations and the availability of patients for investigations. Micro-dialysis is used to look at tissue penetration and works well in superficial tissue and even in lung tissue, but it is certainly not a tool for use in deep tissue like pancreatic and prostatic tissue. We know that taking tissue biopsies, homogenising them and extracting drug provides somewhat misleading information and a direct comparison of serum levels with tissue levels at one time point after administration can give almost any answer you want. Because of that there is significant bias in the literature about tissue penetration. I agree with you that the information is difficult to find. For example, information about glycopeptide penetration into infected lung tissue is at a premium.

Comment from Derek Brown on future funding of EUCAST

EUCAST has been through an ECDC review process. They are reviewing all European funded activities in the surveillance area. Until early next year they will fund EUCAST to the same level as the European Community grant previously agreed, then they will consider integrating it into the surveillance networks which are going to be supported in Europe. ESCMID want to stay involved as a professional organisation and would provide funding.

Susceptibility testing of mucoid *Pseudomonas* and *Burkholderia* strains from patients with cystic fibrosis including evaluation of the BSAC standardized method *Dr John Perry, Newcastle*

Comment from John Perry on susceptibility testing of Pseudomonas isolates

People around the country who do testing on cystic fibrosis isolates feel that this is a neglected area. One possibility is to take large numbers of *Pseudomonas* isolates, including non-cystic strains, from several different centres and test them in different laboratories to see if there is a statistically different trend in susceptibility between cystic fibrosis and non-CF *Pseudomonas*.

There was general agreement in the audience that there is a lack of guidance on testing cystic fibrosis isolates.

Value of throat swabs from infants

Q: A very important point is the value of testing some of the specimens we receive from infants and very young children attending CF outpatient clinics. When a child cannot produce sputum the paediatrician sends a throat swab. How should the laboratory process the sample?

A: If you can't get any other specimen it's difficult to say you don't process it. I think a lot of these tests are done for the clinician's and laboratories' peace of mind rather than the benefit of the patients.

Effect of treatment on susceptibility

Q: Have you noticed whether the MICs increased after treating CF patients with antibiotics?

A: MICs generally don't seem to go when patients are treated. You get a combination of isolates with different susceptibility each time you do the test. There is no consistency. In some you get resistant isolates where you didn't have them before and in some it's the other way round.

Synergy testing

Q: For synergy testing what antibiotic concentrations did you use?

A: We used BSAC breakpoints.

Q: The method that you used is not a conventional FIC test. Do you think that if you had used a conventional method you would have had different results?

A: The method we used is a well published method with a huge number of strains and we just adopted it as was. It would appear to show synergy but we could also get an additive effect or inhibition. I am not sure of the correlation between the traditional FIC test and this method of testing.

Q: have you ever considered using a conventional FIC method?

A: We are using our current method for pragmatic reasons. In one tray we can effectively do 78 different antimicrobial susceptibility tests. We cannot consider using a conventional FIC because it would be impractical.

Q: Do you suggest the therapy that should be used?

A: We strongly believe in combination therapy for *Pseudomonas aeruginosa*. We produce a report of all combinations that produce a 99.9% kill. It is then up to the clinicians to look at the combinations they use, taking into consideration the particular patient details.

Q: Are laboratories linking susceptibility tests to clinical response data? Do they choose an antibiotic and then record what happens in the patient?

A: I can't give any answer with scientific evidence. We get positive feedback from our clinicians, e.g. we have transplanted patients with *P. cepacia* resistant to all antibiotics tested individually and then we put them on combinations and the patients do very well. In Ireland they have some very difficult patients and we have given them recommendations and have got very positive feedback. However, none of this is validated in a scientific way.

Significance of multiple phenotypes and susceptibility patterns

Q: When you have multiple different organisms and susceptibility patterns from a single specimen, which one do you use for synergy testing?

A: We have just completed the work with culturing sputum samples on selective media and, for example, have found 30% of isolates amikacin resistant. We are now facing the question of how we use this selective media method as a routine.

Q: Do you test multiple colonies from the same organism, or do you pick whatever organism you see growing on the plate?

A: One of the problems is what is the right result. We assume that we want to pick out the resistant organism but we don't actually know that that is the case. The presence of resistant organisms doesn't correlate with treatment failure and the presence of susceptible organisms doesn't correlate with treatment success. I think we should start by establishing what is in the sample and then try and correlate that with treatment regimes.

Growth state of infecting organisms

Q: What about the growth state of the organisms, because cystic fibrosis organisms are essentially growing in biofilms. How does this affect testing?

A: I think there are a lot of data showing an altered response of the organisms in biofilms. I think it has been well demonstrated that we have different susceptibility results with organisms in the biofilms. It could be important but is not something we have looked at. As with all this, studies are required to correlate the biofilms susceptibility results with clinical outcome.

Q: Do you think that *Pseudomonas* from patients with cystic fibrosis grow more slowly than organisms from other pseudomonal infections?

A: Yes, I think some of them do. You can get reasonable growth at 24 h, but it certainly doesn't look like the normal fully developed growth you get with *Pseudomonas* from urine samples.

Q: Have you looked at correlation with normal *Pseudomonas* results?

A: There was an interesting report from Nicola Potts and David Livermore in JAC 2004 which reported on the BSAC method. They highlighted problems with some of the same antibiotics, such as aminoglycosides and ciprofloxacin. Some of the problems you see with the disc diffusion method are due to problems with *Pseudomonas* as a whole and some of the problems are with slower growing cystic fibrosis strains.

BSAC recommendations for interpreting the susceptibility of urinary tract isolates Mrs Jenny Andrews, Birmingham

Quinolone susceptibility testing

Q: Would you recommend testing for fluoroquinolone resistance with nalidixic acid?

A: For isolates from urines some laboratories like to screen with nalidixic acid, others prefer to use ciprofloxacin and if they see a reduced zone then maybe go on to do an MIC. It's the choice of your department.

Interpretation when organisms are not identified

Q: For laboratories that don't identify their Enterobacteriaceae, would it be better to use systemic breakpoints and add on nalidixic acid and nitrofurantoin, rather than getting it wrong occasionally with *Enterobacter*?

A: In the footnotes to the tables we are trying to point out that the interpretation is for uncomplicated UTIs and that identification is essential for correct reporting. What you suggest is a sensible and cautious approach.

Interpretation when organisms are isolated from urine and blood

Q: There are occasions when we get a urine result before a blood culture result and the susceptibility to ciprofloxacin may be different. What do you suggest that laboratories do?

A: Using the systemic recommendations might help, but obviously the report will have to be amended.

Methicillin susceptibility of *S saprophyticus*

Q: In methicillin sensitivity testing of *S. saprophyticus* there are invariably enormous zones to ampicillin or amoxicillin and yet they may be resistant to methicillin. How do you recommend reporting the β -lactams in that situation?

A: Resistance to methicillin is very rare in *S. saprophyticus*, but there have been reports of isolates in South America. With methicillin the MIC distribution for the “wild type” population straddles the MIC BP and therefore organisms may appear methicillin resistant. Using oxacillin, where the MIC breakpoint is separate from the “wild type” population, there is no false resistance.

Susceptibility testing new agents e.g. linezolid, tigecycline, daptomycin and dalbavancin *Dr Ian Morrissey, London*

Antimicrobial antagonism

Q: We are aware of inducible resistance with clindamycin and macrolides, but have noticed the same effect with linezolid and ciprofloxacin.

A: You are seeing antagonism between ciprofloxacin and linezolid. I am not aware of anyone studying that phenomenon but the action of quinolones is antagonised by most bacteriostatic agents. My guess is that linezolid is inhibiting protein synthesis and not allowing ciprofloxacin to work effectively. I haven't seen that in the literature anywhere.

VISAs and dalbavancin

Q: Do the mechanisms which give rise to intermediate or heterogeneous resistance to vancomycin in *S. aureus* impact on dalbavancin susceptibility?

A: My guess is that they would because the mechanism is a thickening of the cell wall. Certainly a similar size molecule they would have the same mechanism.

Q: Do you know if there are any MIC data for dalbavancin against VISAs?

A: I would be surprised if it hasn't been done and I would be more surprised if there was activity.

Q: With dalbavancin do you get cross resistance with VanA but not VanB mediated resistance in enterococci?

A: Yes that's right.

Comment from Derek Brown on licensing of dalbavancin in Europe

Dalbavancin is being submitted to the European Medicines Agency for a licence in the next month or two, after which a breakpoint will be set.

Comment from Jenny Andrews on linezolid susceptibility testing

We noticed different results between laboratories in linezolid MIC tests in a Working Party study. Three centres tested the same organisms by MIC; two laboratories had the same results the other consistently higher. This was the reason for raising the breakpoint for staphylococci.

Dalbavancin susceptibility testing

Q: Is the reason for adding Tween 80 to tests on dalbavancin to avoid it sticking to plastic surfaces?

A: It is a similar molecule to teicoplanin, sticking to plastic and molecules clumping together. What the CLSI recommend is that you not only have the Tween 80 in the wells but also as a component of the diluent. The compound is dissolved in DMSO and Tween 80 is added to the diluent.

Daptomycin susceptibility testing

Q: Is it possible to determine daptomycin susceptibility using disc diffusion testing?

A: Disc diffusion testing is not recommended because it is difficult to differentiate between organisms with MICs close to the breakpoint and those that are susceptible. Chiron looked at discs containing calcium as well as daptomycin for use with Iso-Sensitest agar, but results were disappointing. It was also noticed that Mueller-Hinton results varied depending on the batch of medium used for testing. With disc diffusion testing the difference between the susceptible population and those with low level resistance is only a couple of mm and the concentration of calcium in media makes a difference. This is also seen with MIC testing where a reduction in calcium content lowers the MIC by one or two dilutions.

General questions and answers

Tigecycline susceptibility testing

Q: We took part in the national tigecycline susceptibility survey, with disc diffusion testing and Etest MICs. The majority of Enterobacteriaceae by disc diffusion testing tended to fall into the intermediate category but by Etest they were susceptible with tigecycline MICs around 0.5 mg/L. Are the disc testing recommendations correct?

A: If you would send the data and the organisms to Jenny Andrews the BSAC can look at them. It could be a problem with the breakpoints or the way that you do the test. To have someone else check it is worthwhile. It could be also an issue with the E test and a reference method would be a dilution method of some description.

Comment from Derek Brown on breakpoint setting

Breakpoint setting is not as precise a science as we would like. Sometimes breakpoints are shifted (as we have mentioned today already) just so they don't create problems with normal distributions. It is not unheard of for breakpoints to be changed when there are sufficient data to support a change in breakpoint. When breakpoints are set they are done so on the information that is available at the time.