

## Sheffield User Group Day Tuesday 18 October 2005

### Morning session

#### Most often asked questions

##### Jenny Andrews

**Q.** Jenny, referring to your comments about meropenem and the *P. aeruginosa* control, we are having similar problems in our laboratory and have sent our control strain to Oxoid for investigation. Oxoid say that when they use their control strain and discs their zones fall within the BSAC acceptable limits, whereas we are constantly finding that we are at least a couple of millimetres above the limits. Can you suggest why that is?

**Q.** (Jenny Andrews). Where do you get your media?

**R.** Oxoid.

**R.** (Jenny Andrews). Variation in performance can be attributed to media, discs and also the control strain used. We have all seen problems with media and discs, but often we do not suspect the control strain. When there is a problem I think we should also check the performance of the control strain, particularly if it is from a commercial supplier, against an organism obtained from the National Collection of Type Cultures.

**R.** Oxoid suggested that we were storing the discs incorrectly. However, the conditions under which we store discs have not changed and we adhere to the BSAC recommendations. I don't see what else we can do.

**R.** (Jenny Andrews). I will pass on your remarks, and any data to support your observations, to Oxoid for their comments.

#### Cefoxitin for the detection of methicillin/oxacillin resistance in staphylococci

##### John Perry

**Q.** When you say that the BSAC are now recommending cefoxitin for detecting MRSA, is that instead of methicillin as we are currently doing both methods in parallel?

**R.** (John Perry). The January 2005 guidelines say that cefoxitin tested on ISTA can be used on its own as an option to determine methicillin resistance in *S. aureus*. Alternatively, Mueller Hinton or Columbia media plus salt can be used to test methicillin or oxacillin.

**Q.** What MIC corresponds to a zone size of 20 mm for the cefoxitin 5 µg disc?

**A.** (John Perry). 4 mg/L.

**Q.** If you wanted to incorporate cefoxitin into agar for MRSA screening purposes what concentration would you use?

**A.** (John Perry). A cefoxitin concentration of 4 mg/L. There are different methods used for isolating MRSA. Oxacillin and ciprofloxacin have been added to agar, but from our studies in Newcastle we have found that the addition of cefoxitin to screening agar gives excellent results. It should also be noted that cefoxitin is incorporated in 2 commercially available MRSA media for the isolation of MRSA.

**Q.** We trialed Bio-Rad media for detecting MRSA and found that susceptible *S. aureus* (by Etest) were growing on the media.

**R.** (John Perry). I think one of the problems is that media incorporating a concentration of 4 mg allows susceptible isolates to grow particularly when plates are close to the expiry date. Unfortunately, the concentration cannot be increased to 6 mg/L because you start losing the odd MRSA. We have seen this with other media.

## General questions

**Q.** Organisms we often have difficulty with are the respiratory Pseudomonads that don't grow in the time scale specified for a BSAC disc test. Is there any recommendation for testing these isolates?

**R.** (John Perry). It is certainly a problem in our laboratory. We have strains that don't grow for 48 hours and then how do you interpret their susceptibility tests. Cepacia is an area that the BSAC are going to have to look at in future.

**Q.** What do you do with *Burkholderia* spp. Do we use the recommendations for Pseudomonas?

**A.** (John Perry). I don't think enough studies have been done to say whether that is correct. I would suggest undertaking an MIC, possibly an Etest.

If we have multiresistant isolates of *Burkholderia* and *P. aeruginosa* we do routine synergy testing perhaps 67 different combinations of antibiotics. We get referrals from laboratories around the country and we often find there are discrepancies with disc susceptibility testing and the MIC results. I do think it is an area that needs a lot more work to develop a reliable disc susceptibility testing method.

**Q.** We have had a couple of problems during the last two weeks with a *S. pneumoniae* isolated from a patient with an eye infection, on primary testing it was susceptible to tetracycline. When the test was repeated as an IQC it was resistant to tetracycline. Would you expect this organism to be resistant to tetracycline? Also is *S. pneumoniae* with reduced susceptibility "resistant" to tetracycline?

**A.** (Jenny Andrews). Tetracycline is fairly easy to test because it a bimodal distribution, so I think there is something strange about the results. Are you sure that the same organism was tested on both occasions?

**C.** (Alasdair McGowan) Around 2-3% of pneumococci are tetracycline resistant. The UK, like other parts of the world, isolates with reduced penicillin susceptibility are more likely to have other resistances, so the occurrence of reduced penicillin susceptibility and tetracycline resistance and maybe macrolide resistance are not too surprising.

**Q.** In the past we have disc tested *S. pneumoniae* against cefotaxime. In the latest recommendations it suggests that an MIC such as Etest should be undertaken, is this necessary?

**A.** (Jenny Andrews). In CNS infections the BSAC recommends that susceptibility to cefotaxime and ceftriaxone is determined by an MIC. If you have an organism with reduced susceptibility to penicillin based on oxacillin disc testing, a penicillin MIC should be undertaken to determine the level of resistance to penicillin.

**C.** (Alasdair McGowan) Isolates of *S. pneumoniae* with high-level resistance to penicillin are not very common. There is some evidence, through the surveillance of resistance surveys that penicillin non-susceptible pneumococci are declining in the UK and also to a marked degree in Southern Ireland. The greatest impact is in Liverpool and Glasgow, where resistance rates lie somewhere between the UK and Southern Ireland average. When resistance occurs, it is important to confirm the level of resistance by MIC i.e. Etest, because the vast majority of strains in the respiratory tract will be treatable with high dose amoxycillin.

**Q.** Clarithromycin and Haemophilus infections, isolates always have intermediate susceptibility?

**A.** (Alasdair McGowan). That's the right answer. What we mean by intermediate is indeterminate, because we don't know what the clinical response is. Breakpoints that have been determined by the BSAC force laboratories to interpret all Haemophilus as having intermediate susceptibility to clarithromycin. Treatment of *H. influenzae* infections with a macrolides, erythromycin and clarithromycin and maybe also azithromycin (which is not commonly used in the UK) has an uncertain therapeutic outcome. CLSI have decided Haemophili are susceptible, many Scandinavians don't know or consider that they are resistant. The BSAC therefore gives a conservative answer.

## Afternoon session

### Antibacterial drug development

Alasdair McGowan

**Q.** Is there any truth in the media stories of deep-sea squid and crocodiles killing MRSA?

**A.** (Alasdair McGowan). Generally, no. A gentleman who is interested in alternative medicine brought back some Kalama tree bark from India. We did some tests and found that it had cidal activity against MRSA. The issue is determining the active ingredients in alternative medicines (berries from the tree etc) and finding out whether they are safe for humans as well as being lethal for bacteria.

**Q.** Should patents be changed from 10 to 20 years and from national to international patents?

**A.** (Alasdair McGowan). I don't know who decided on the length of patents, but primarily they were set up with Europe and the USA in mind, so I assume was done internationally. Patents were a way of ensuring drug development and the situation would be enhanced if intellectual property patents were improved.

**Q.** When a drug comes off patent and another company starts making it, are there any checks to see if the drug composition is the same as that made under patent?

**A.** (Alasdair McGowan). In Europe there is sufficient regulatory control to ensure that this happens. However, if you go to South America, Mexico or India and take a ciprofloxacin tablet for example, the tablet may not contain the correct ingredients. In the case of moxifloxacin, it was being copied in India by generics houses long before Europe and North America.

**Q.** If you think you are taking 500 mg of a drug and in actual fact you are having 250 mg, could this have an impact on the emergence of resistance?

**A.** (Alasdair McGowan). Yes, but emergence of resistance could also be associated with easier and wider availability of antibacterials, without medical or pharmaceutical prescription.

### Expert rules and inexpensive identification methods

Trevor Winstanley

**Q.** Trevor are you going to cut down the number of tests for the ID set or have you done so already?

**A.** (Trevor Winstanley). Currently, for expert rules, we use 15 biochemical tests. We might introduce a Chromogenic agar plate and then we can reduce the number of tests to 6.

**Q.** Does the list of organisms that you showed cover AmpC producers?

**A.** (Trevor Winstanley). It is fairly comprehensive for those organisms that are looked at in the laboratory. In Gram- negatives, if cefoxitin resistance is shown then organisms have AmpC or there is a low chance that it has lost the outer membrane porin. If AmpC production is suspected a cephalosporin or a  $\beta$ -lactamase inhibitor combination (e.g. piperacillin/tazobactam, co-amoxiclav) should not be used clinically.

*E. coli* have a very low level of chromosomal AmpC, but *Klebsiella* spp. do not, but both can receive *ampC* on plasmids from *Enterobacter* spp. and *Citrobacter freundii*. At the Hallamshire we do direct sensitivity testing on blood cultures, and include cefoxitin. If the organism is resistant to cefoxitin the clinicians know not to treat the patient with a cephalosporin or piperacillin/tazobactam.

**Q.** Is it useful to report AmpC?

**A.** We report ESBLs and AmpC. If derepressed, the clinicians won't treat with a cephalosporin. We also apply expert rules to the result and for *Enterobacter* spp. We

amend piperacillin/tazobactam to resistant and insert the following comment to the report "*This organism may fail cephalosporin therapy*".

**Q.** David Livermore produced a table that said that certain phenotypes were always resistant to piperacillin/tazobactam. Is this so?

**A.** (Trevor Winstanley). That is correct. The table was originally for ESBLs and stated that, in serious systemic infections the advice is not to use piperacillin/tazobactam because the enzyme overwhelms it.

**Q.** For ESBLs that we isolate we are finding quite a few with borderline zones to ertapenem. Have you any thoughts on that?

**A.** (John Perry). This was discussed at our last working party and certainly I had noticed the same thing. I collected together 76 different coliforms that included groups of various species with different mechanisms of resistance, and did MIC tests and disc testing as recommended by the BSAC method. We currently have a zone diameter breakpoint of 34 mm. It seems from the data that I gathered, backed up to some extent by HPA Colindale, (David Livermore's group) that the breakpoint is much too high when you look at the corresponding MIC breakpoint. Certainly anything that had a zone size above 22 mm is susceptible to ertapenem. There are few strains that are genuinely resistant to ertapenem, so we need to gather together a collection of resistant isolates in order to reliably identify a new zone diameter breakpoint. The current breakpoint is too high, we agree on that and it will be changing in the near future.

**C.** (Alasdair McGowan). There are ESBL producing isolates that have cefotaxime and ceftazidime MICs that range from 1-2 to 64 mg/L. The strains with the higher cephalosporin MICs also have a shift in their ertapenem susceptibility; with MICs up to 0.25mg/L, close to the present breakpoint of 1mg/L. It has been argued that the breakpoint ought to be 0.5 mg/L. I think there is a broader question as to what the activity of ertapenem is against some of these strains that are producing a lot of enzyme, particularly in circumstances where you have a large inoculum e.g. an intra-abdominal abscess.

I think we have to take into consideration that ertapenem is less active against an organism that is AmpC stably de-repressed. Clinical response data for patients treated with ertapenem is needed, particularly for *Enterobacter* spp. and ESBL producing bacteria (currently not available in the trials database). Data that is available is for imipenem, the widest used carbapenem in the USA, where these isolates are commonly isolated. When you look at the clinical data in isolation there is not a lot to support ertapenem.