BSAC
british society for antimicrobial chemotherapy
11 The Wharf, 16 Bridge Street, Birmingham B1 2JS
Tel: +44 (0)121 633 0410 Fax: +44 (0)121 643 9497
Email: enquiries@bsac.org.uk Website: www.bsac.org.uk Charity No: 1093118 VAT No: 442 5855 40

Standardized Disc Susceptibility Testing Method User Group Meeting

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QUESTIONS AND ANSWERS

Veterinary susceptibility testing

Speaker: Mr. Chris Teale, Veterinary Laboratories Agency

Have there been any studies specifically looking at levels of antibiotic resistance within meat products?
CT. There has just been an FSA study on chickens and the results are available on the FSA website. Traditionally in the VLA we have tended to stop our surveillance at the point of the abattoir and we haven't done much on food. The big study I know of is the FSA study on farm chickens and I think there have been some studies on eggs as well. I agree there is a bit of a gap and we could do more on food.

Looking at your presentation, you have tested strains from different animals some of which would have different body temperatures. Is that something you take into account with your testing techniques, some are I imagine more thermophilic?
CT. We don't, but maybe it is something we should do. I know the body temperature of a chicken is higher than that of a mammal and that is supposed to be the reason why thermophilic Campylobacters grow so well in birds. We don't have any differential testing apart from for fish and Campylobacter – all fish work is done at 20°C, consequently it can take ages to get a result back for fish.

The third generation cephalosporin resistance in* Salmonella newport *was that Extended* b*-lactamase mediated, do you know?
CT. No, in Newport in the States it is related to CMY2 so it is an AMPC β-lactamase, not an ESBL. The presence of ESBLs or AmpCs in veterinary Salmonellas in the UK would be a significant development for us; we have only ever found one Salmonella that had AmpC resistance or ESBL resistance to third generation cephalosporins and that was a single isolate of *Salmonella bredeney* that had the AmpC gene CMY2 and in that case there was a link to an imported animal. We are keen to maintain our good figures on third generation cephalosporin resistance in Salmonella as far as we can.

Can I ask Chris, do you look at the relationship between infections that say a pet gets and the owner might get from the pet and how antibiotic resistance patterns might affect the treatment of the dog or cat or the patient?
CT: We tend not to do much companion animal work at the VLA. There has been a lot of discussion recently about MRSA in pets (cats and dogs). Most of the recent UK isolates reported in the literature are from dogs or cats, although one was recorded from a rabbit and one from a horse. Most of the published reports of MRSA in animals have been from abroad but there have been two recent studies in the UK, one done in Yorkshire at a private lab (Idexx) and one done at the RVC (Royal Veterinary College, University of London) and they have found that many of the isolates were recovered from wound infections of animals that had recently undergone surgery, which starts you wondering whether carriage of MRSA by the owners or the veterinary surgeons is leading to infection of these wounds. Now that you are getting much higher rates of community infection with MRSA in humans, does that mean a spill-over into the animal population is more likely and that MRSA isolates may start to be associated with things like ear infections or urinary tract

infections in small animals? The big question is what is the origin of MRSA found in animals? Animals may not be important as a significant source of MRSA for humans, but maybe they can act much like fomites. As to the pattern of antibiotic resistance in small animals, most small animals are treated once for a condition or if they have a chronic infection such as an ear infection they might have ongoing treatment, but there isn't usually the pressure in veterinary hospitals that you have in human hospitals, with things on a continuous through-put or with tremendously immunosuppressed patients. Those cases that are intractably difficult to treat I would say are more often euthanased, they may not get to an ongoing, very chronic stage. May be that is why we haven't had problems yet in veterinary practices that occur in hospitals.

It is an interesting point Chris, because we have been looking for associations between antibiotic resistance and various things such as prescribing practice and hand washing in the hospital environment. Perhaps we should also be looking at animal vectors as a means of getting resistance to our population of bugs.

CT. For food producing animals there are authorised treatments that you can use –authorised drugs which have been fully tested in that species; then there are very limited exemptions to that, so if you had a species which was also producing food but for which there was no licensed treatment available, then a vet could use another drug which was also licensed for use in a food producing animal but in a different species. You are not allowed to use advanced medical antimicrobials like imipenem, carbapenem or isoniazid in food-producing animals; all those things would be prohibited. But in a dog or cat or animal that is not going to be consumed a wider range of authorised veterinary medicinal antibiotics is available for use under prescription. The authorised medicines for treating dogs and cats are almost universally of "older" antimicrobials than many of those currently used in human medicine and they would comprise by far the bulk of treatments administered.

Methicillin/oxacillin resistance in staphylococci

Speaker: Dr. Derek Brown, Addenbrooke's Hospital, Cambridge

Dr Brown in your talk you mentioned coagulase negative staphylococci, did you actually say that you don't need to incubate them for 48 hrs to pick up methicillin resistance?

DB: We are not doing that. For some of the resistant strains incubation for 48 hr does improve the expression of resistance and you can see colonies within zones more clearly, but we also find that at 48 hr many of the susceptible strains give smaller zones, some below the breakpoint. Hence it may make it easier to see some of the resistant colonies within zones, but it is also likely to increase the false resistances. I think it is important to look at the zones carefully although I know it is more difficult for a routine laboratory. Very often our staff read plates very quickly and I am pretty sure you have to look closer to pick up some of these resistance mechanisms. If you look closely at these zones you can see the colonies within the zones, so the key is to look at the zones closely at 24 hr rather than the 48 hr.

You also suggested that cefoxitin testing might be an alternative to methicillin and oxacillin testing. Have you done an extensive survey and do you see small colonies in the zones?

DB: We have done a large study. You do get the same sort of effects with cefoxitin as with oxacillin with some strains – it is not clear cut. Different groups have had different experiences with cefoxitin on Iso-Sensitest agar. The Swedish group say that they get good results and we say that some strains are missed at 37°C, but it may be acceptable at 35°C. The other point is that cefoxitin at 37°C on Iso-Sensitest does not work for coagulase-negatives - we'll miss a substantial number of resistant isolates at 37°C. On the other hand, there is no doubt that testing with cefoxitin solves the problem of the penicillinase hyperproducers. I think there will be a good argument for replacing the oxacillin with a cefoxitin disc and we may be producing some guidelines on that before long, but that we need to spend a bit more time on this. I should also mention that cefoxitin production may be stopped - some of the generic producers may continue with production, but we don't know if that is the case. Cefoxitin is a cephamycin antibiotic and there are some publications suggesting that other cephamycins such as moxalactam might work also, but there isn't the data to support that in disc diffusion testing.

Back to reading staphylococci; you have to reinforce that you must leave things for 24 hours, that is the important thing, rather than rushing them out of the incubator.

DB: I do say that, and it is related to picking up the small colonies, if you incubate that extra few hours the small colonies within zones do become significantly easier to see. The key is actually spending the time to look at the zones carefully.

Can I question the practicalities of that in your own laboratory; do you leave the reading of methicillin to later on?
DB: We leave them until last when reading. Most of our tests get set up late morning or around lunch time and are read first thing in the morning so the oxacillin tests probably do not get a full 24 hours. We have to be pragmatic, but certainly you don't want a situation where you set up the tests at 5pm in the afternoon and read them at 9am the next morning. Some of the resistant community strains that are turning up can be quite difficult to see in less than 24h.

Derek you mentioned testing resistant organisms with latex agglutination for PBP2a when the zones were just resistant. How many of yours do you do, is it a frequently done thing?
DB: In reality it is very few and it is subjective as to whether they get done or not. I would suspect that we report some as resistant that are actually hyper- producers of penicillinase. We have never done a study to see how frequently this occurs in Cambridge. Probably we do not do more that one a week. I can add to that, in our own experience, we do exactly that sort of methodology, but I can't define how often, I don't know whether my colleagues here today could.

Can I ask how we might demonstrate hyper β-lactamase producers?
DB: If you have got small zones with MRSA you tend to still get fading of colonies into the zone whereas with the beta-lactamase hyper-producers you tend to get a small zone with quite sharp edges, you don't get that fading of colonies which is so common with that type of MRSA.

I don't think there is any standard definition. If you put a co-amoxyclav disc near an oxacillin disc you do get potentiation of the activity by the co-amoxyclav but it is not a realiable definitive test. In fact, I am not sure there is one because if you look at the whole population of *Staph. aureus* there is a complete range of production of beta-lactamase from some that are very weak producers all the way up to those that are very high level producers. So I think it is a fairly arbitrary description. Certainly the strains we included in our study were significant high-level producers because they give almost no zones with oxacillin in some tests.

We use the Mastalex test in our laboratory rather than doing direct sensitivity tests on blood culture isolates and so on. We use it for Staph aureus isolates and occasionally we seem to have had a false positive in that we have had a positive reaction on the Mastalex and when we did the methicillin disc test to confirm we get a large zone. Have you ever come across any false positives or if you had a situation like that would you put more faith in a Mastalex or in your methicillin test?
DB These are direct tests you are doing from agar plates and you repeated them and you still got positive results with Mastalex and negative on disc testing? I haven't seen any reports of that and it might be worth asking a reference lab to confirm resistance.

Recent changes to the recommendations
Speaker: Mrs. Jenny Andrews, The BSAC Standardized Method Development Centre

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You mentioned recommendations for anaerobic organisms, which filled me with some trepidation. Will the recommendations be species related and are we going to have to spend a great deal of time identifying anaerobes, which I know are done fairly poorly being close at hand with the anaerobe reference laboratory here in Cardiff?
As with all susceptibility testing, identification aids the recognition of mechanisms of resistance and the application of expert rules. For anaerobes that grow feebly after overnight incubation, there is a poor relationship between MIC and zone diameter so identification is necessary as the BSAC will only be giving recommendations for fast growing anaerobes such as *B. fragilis* and *C. perfringens.* For other anaerobes an MIC determination will be recommended.

Talking about slow growing organisms, we have a problem with cepacia.
The BSAC recommendations unfortunately do not include this group of organisms, so I suggest that you do an MIC.

GENERAL DISCUSSION

We seem to have a cluster of Staphylococci that will only grow in CO_2; can you recommend how we interpret results?
DB I have never come across them, perhaps you could send some to us.
Could I emphasise that if you have a strain that takes 48 hr to see visible growth you shouldn't be doing a disc diffusion test. Like any organism, if it takes that long to grow you are inevitably going to get very large zones, even with strains with elevated MICs

Does the same argument apply to Etest?
DB No, it doesn't seem to be quite the same with Etest with which tests have even been done on rapidly growing Mycobacteria. I think it is something to do with the time the Etest gradient stays stable being much longer than the time the disc diffusion gradient is stable.

Going back to the Stenotrophomonas when you had the testing at 30°C everything greater than 20 mm is sensitive. What actual antibiotics were you talking about?
JA. Just cotrimoxazole. As I mentioned, for β-lactams and ciprofloxacin MIC results are variable depending on medium and temperature used for testing and there is no good correlation between MIC and zone of inhibition. The BSAC therefore suggests that only cotrimoxazole is disc tested. If you look at the website you can find the updated recommendations.

We have a patient coming in with it regularly, so should we send it off for MIC testing?
JA. No I suggest you contact Robin Howe a Consultant Microbiologist at Southmead Hospital in Bristol for clinical advice.

The BSAC information we have says that we should only use this drug for Pneumocystis carinii
JA. I think if you look at the wording it states that cotrimoxazole ` *can be used in specific instances*', and this is a specific instance where cotrimoxazole would be appropriate.

Every time we test Haemophilus to erythromycin all isolates are resistant, for clarithromycin organisms are either moderately resistant or resistant. Should we be incubating tests in CO_2 as the method suggests?
JA. The acid environment produced when tests are incubated in CO_2 adversely affects the *in-vitro* activity of macrolides. Erythromycin MICs for *H. influenzae* undertaken in the presence and absence of CO2 are markedly different, around 4mg/L in CO_2 and 0.25 mg/L in air. The BSAC suggests incubation in CO_2 firstly, to ensure that haemophilus grow confluently after overnight incubation, and secondly, the research work undertaken at Birmingham University by Sue Hill *et al* indicates that the pH within the respiratory tree is acid, around pH 6. As the concentration of erythromycin in the bronchial mucosa is approximately 3 mg/L, which is lower than the MIC for the `wild sensitive population', it is not surprising that most isolates are resistant. For clarithromycin there is greater penetration in the respiratory tree and therefore isolates may be more susceptible to clarithromycin.

***I don't know whether anyone else has noticed but some of our synercid zone sizes seem to be getting slightly smaller. We have sent one or two of them off and what they say to us is the MIC of the synercid is raised but still remains susceptible. Do you recommend if we get something like that we should be using a more sensitive method to check them out? This is related to synercid against* E. faecium.**
JA. I am surprised that you had those results with *E. faecium* because they are normally susceptible. Are you testing on blood?

No on plain Iso-Sensitest agar. The susceptible zone for synercid is 20 mm, but with perfect inoculum we get zones of 17/18 mm that makes them resistant. Reports come back with 'raised MICs but still susceptible'. So should we do an Etest to confirm.
DB: If the reference laboratory says they have reduced susceptibility but MICs are lower than the susceptible breakpoint, we would still call them susceptible. I don't know how the Etest works for these organisms, but maybe you could try it yourself and see if it agrees with the reference laboratory. It would be a lot easier to check them yourself.

Are you recommending nalidixic acid as the agent for testing ciprofloxacin for all organisms?
JA. It depends on the organism, if you were testing *H. influenzae* it is possible that low-level resistance would be missed if a ciprofloxacin disc were used. For Haemophilus and *Neisseria gonorrhoea*, Campylobacters and invasive Salmonella a 30 µg nalidixic acid disc is recommended by the BSAC for detecting resistance. In the

case of UTIs there is a difficulty because using only nalidixic acid would mean that *E. coli* with low level resistance, that would probably respond clinically, would be reported resistant

Glycopeptide testing in S. aureus by E test. I believe the recommendations are to test using brain heart infusion agar and a 2 McFarland standard inoculum. This is what we are doing, but we get a fair number of organisms falsely resistant, which I believe is well recognised. The advice is to check those by population analysis.
DB. That is exactly it. The Etest method as described is a great method for picking up heterogeneous resistance, but the definitive test is population analysis

Chris one of the items raised in your talk implied that the source of human infection may not be food chain.
CT. We only test animals from England and Wales and we didn't have any Salmonellas with those patterns that were occurring in man, suggesting that those Salmonellas were coming from another source.

Do you test all Salmonellas from livestock in England and Wales?
CT. I think we test as many as we can, as it is a legal requirement that they are sent to us for testing.

Is it useful to undertake electron microscopy on suspected VISAs?
DB. Doing EM on sections of Staphylococci is considerably more difficult than doing population studies on VISAs.

Derek, in your talk you said that the NCCLS are coming more in line with the breakpoints used by the British. Can you expand on that?
DB: There is a lot more critical examination of the older breakpoints now. There is a lot more data available that wasn't available when some antibiotics were first on the market. Jenny mentioned EUCAST and susceptibility testing. Within that group all the active breakpoint groups in Europe like the BSAC, and the French, Swedish, Norwegian, Dutch and German groups have agreed to a process whereby we re-examine all of the breakpoints for all the antibiotics. It is going to take a little time but using the new approaches, including pharmacodynamic data available now, they will try to come to some agreement on breakpoints, at least in Europe. The EUCAST chairman, Gunnar Kahlmeter, has been talking to NCCLS and some members of NCCLS are open to the idea that they may have got some of the breakpoints wrong (some of them being too high). There is a great reluctance to change the breakpoints, as there is a lot of commercial influence at NCCLS that resists change. Pharmaceutical companies do not want their breakpoints reduced as it might make their products appear less active. The device manufacturers are against changing breakpoints as it means they have to change their systems. The NCCLS are re-examining some of their breakpoints and in fact have started looking at the cephalosporin breakpoints for Gram negative organisms, many of which we find very high. I think ESBL-producing strains have bought it home that they need to look again. I think it will take years before there is international agreement between us but the general trend is in the right direction.

Sometimes there is only one dilution difference between countries in breakpoints and it can often be argued that breakpoints should be one dilution up or down - sometimes the BSAC will have to move breakpoints to get consistency throughout Europe. I think the success in Europe in getting the groups together in EUCAST has been noted by NCCLS and at least some members of NCCLS are interested in discussions with EUCAST.

I wanted to ask Chris if antibiotic resistance in animals is driven by antibiotics in food and growth promoters and with the regulation of this have you seen any decrease in resistance rates and if so, how long has it taken to see a decrease?
CT: I suppose I should give a bit of background on growth promoters. A lot of these were banned in 1999 because they were related to or caused cross resistance with human antimicrobials, for example avoparcin showed cross resistance with vancomycin. Virginiamycin, the macrolides tylosin and spiramycin and zinc bacitracin were all banned throughout Europe at this time. There are currently four remaining growth promoters -salinomycin, monensin (which are both ionophores) avilamycin and flavomycin, and these are all due to be banned in January 2006 throughout Europe. So there will be no growth promoters used in Europe following that ban. The best data available is from Denmark because they have produced the best antimicrobial consumption figures and best monitoring of the changes, particularly in enterococci, that were occurring in their country. All the growth promoters have a Gram-positive action and they don't act against most Gram negatives. Looking at the enterococci it was very interesting as it flagged up the other thing I talked about. The plasmid conferring resistance had three linked resistance genes on it. The Danes banned avoparcin first and the resistance levels in the animal enterococci to glycopeptides went down for example in broilers, but it was only when they later stopped using tylosin as a growth promoter that it went down significantly in pigs. We have recently completed two abattoir surveys in the UK, one in 1999 and one in 2003 and it will be interesting to see whether there has been a decline in vancomycin resistance in enterococci over this period.

In pigs from England and Wales, in the organism *Actinobacillus pleuropneumoniae* (which is a cause of pneumonia) the levels of resistance to tylosin have declined since growth promoters were banned in 1999. I think the Danes in their first two years of banning avoparcin had a decline of more than 50% glycopeptide resistance in their enterococci from broilers, so it is quite substantial, quite an effective measure. However, if we ban use of all antimicrobial growth promoters in the EU but then continue to import from third countries that haven't banned them, we haven't really protected our population, but we have put our farmers at a disadvantage on the global market.

S. saprophyticus*, is there a resistance to methicillin in this group of organisms?*
DB: *S. saprophyticus* is one of the groups that have reduced susceptibility to methicillin as an inherent characteristic. With acquired resistance due to *mecA* in staphylococci you can push up the resistance by selecting the highly resistant sub populations. As low-level resistance in *S. saprophyticus* is an inherent resistance, rather than *mecA* based, you don't have that selection. I presume you are talking about urinary tract infections. As long as you can exceed the MICs you will probably be successful with the treatment given the high concentrations of β-lactams you get in urine

A point about the changes in the updates that you said some people were irritated by. Because it is dynamic system there will be these frequent changes. What in effect it has done, is to change the categorisation of some organisms from sensitive to resistant and therefore the interpretation overtime will change because the criteria have changed. How do you suggest we deal with that?
JA: I suppose the ideal would be to keep zone sizes and then it would be possible to re-look at data, but because laboratories use templates I don't know a way round it.

DB: I don't think there is a way round it unless you have detailed data like MIC data. If you are working on a routine disc diffusion test recording S and R you are going to get changes. If you have zone sizes you can reinterpret.

End of Day