

Sheffield User Group Day October 2006

Members of the BSAC Working party on Susceptibility Testing present:

Trevor Winstanley (Meeting Chairman) [TW]
Jenny Andrews (Speaker) [JA]
Robin Howe (Speaker) [RH]
David Livermore (Speaker) [DL]

Confirmation tests for the detection of AmpC

Speaker: David Livermore

Q: For organisms with inducible AmpC, for example Enterobacters, how should cephalosporin results be edited?

DL: Where an Enterobacter is isolated from a patient with a severe systemic infection it is probably best to warn the clinicians that cephalosporins are inappropriate. Do not edit the results as this will ruin the surveillance data.

Q: How do you report the susceptibility of inhibitor combinations to ESBL producers?

DL: Record the result at face value, but recommend to the clinician that carbapenems have best providence particularly in severe infections. There is not good substantive evidence for editing results of inhibitor combinations specifically for ESBL producers.

RH: There is no evidence based answer to this question. There is no reason why an inhibitor combination shouldn't work against ESBL producing organisms, but there is sparse clinical data to suggest that outcomes are not particularly good and the activity of the carbapenems is just so much superior. I would therefore recommend that reports suggest that clinicians use carbapenems.

Regarding laboratory data and the effect of editing reports on surveillance, in Wales we use the Phoenix system and store raw data before it is subjected to interpretative criteria and edited data. Using this system we have both pieces of information and surveillance data are not affected.

TW: In Sheffield our ceftazidime and cefotaxime resistance rate in Enterobacteriaceae soared during the last year. The reason for this was that we were screening our urinary isolates against cefpodoxime and to work out if these were CTX-Ms or TEM ESBL producers, we were testing them against ceftazidime and cefotaxime, the fact that we were only testing resistant isolates screwed the surveillance data. Be aware.

Q: Can mecillinam be used to treat infections due to ESBL producers?

DL: The Licence in the UK is for urinary tract infections. MICs for ESBL producers and AmpC derepressed strains are commonly low, but there is no clinical data to support mecillinam being used to treat these infections, although it is rumoured that the company may be prepared to do

clinical trial. A 4/5 yr old poster not peer reviewed, stated that ESBL producers and *E. coli* with AmpC were no more resistant to mecillinam than organisms with classical TEM enzymes present. The conclusion was that mecillinam should be effective in these infections, but more work is needed.

Q: Our current ESBL screening procedure is to use cefpodoxime would you recommend this?

DL: Data from London and SE has shown that one hospital only testing cefpodoxime found that half of the cefpodoxime resistant organisms had no substantive mechanism of resistance and the other laboratories found that 2/3 had no substantive mechanism.

Sensitivity screening with cefpodoxime is very good. If there is an AmpC or ESBL it will be cefpodoxime resistant, but the specificity is rather poor, because hyper-production of classical TEM enzyme gives a cefpodoxime-resistant result. Much better specificity is achieved if both cefotaxime and ceftazidime are tested, but I know that laboratories are unwilling to test both agents routinely, particularly for urinary isolates.

Q: The cefpodoxime zone diameter breakpoint has been reduced to 20 mm down from 26 mm and the BSAC guidelines state that organisms with zone sizes between 20-25 mm may have a substantive resistance mechanism. Is the BSAC recommending that organisms with zone diameters of 20-25 mm are tested for the presence of ESBLs?

JA: The data from the study in the South East, that David mentioned, revealed that a high proportion of organisms that were cefpodoxime resistant, were ESBL negative when confirmatory tests were undertaken. Derek Brown and the SMDC looked at current isolates and found that if a zone diameter breakpoint of 26 mm was used 25 % of isolates were ESBL negative. The zone diameter breakpoint was therefore lowered to 20 mm. In our laboratory we tend to get mainly no zone of inhibition or zones greater than 20 mm and check any cefpodoxime resistant organisms by MIC.

DL: This shows the difficulties in this area. Using a cut off of 20 mm reduces the number of 'false-positive' screening results; however, within the zone range of 21-26 mm there will still be a few ESBL producers.

Q: We have had some strains of *E. coli* that have cefpodoxime zones of >20 mm where ESBL production is confirmed.

DL: I think it depends on which particular ESBLs you've got. The prevalent problem in the UK is now *E. coli* and Klebsiellae with CTXM 15 and I agree with Jenny that those isolates give you no zone to cefpodoxime, they are very clear cut. However, you have floating around a few strains that have TEM mutants, some of which are most active against ceftazidime, not very active against cefpodoxime and cefotaxime and can be trickier to detect.

Sensitivity Testing Anaerobes

Speaker: Jenny Andrews

Comment from Val Hall [VH], Anaerobic Reference Laboratory in Cardiff:

When testing anaerobes make sure that plates are put immediately into the anaerobic cabinet and ensure that the cabinet is working properly. There are many incidents of laboratories failing to identify anaerobes because plates have been left on the bench for long periods of time before incubation.

Commercial kits are poor at identifying anaerobes and the reference laboratory now only uses molecular methods. The Reference Laboratory is very small and at the moment is inundated with work on *C. difficile*, so are unable to accept all organisms for typing, but will try to help if organisms are resistant to metronidazole. At the moment the Reference Laboratory uses Etest to determine MICs, but in future intends to batch test by conventional agar MIC methods, possibly at the Antimicrobial Reference Laboratory that has recently been established in Cardiff.

JA. Val do you have any idea of the level of resistance for the commonly isolated anaerobes and what do you suggest laboratories do with organisms that are resistant by BSAC disc testing?

VH. The Reference Laboratory receives organisms for further investigation in a very haphazard way so there is no precise data, although a small amount of metronidazole resistance has been seen.

With regard to organisms resistant by BSAC methodology, these organisms should be sent to the Anaerobe Reference Laboratory for further investigation. There is a need for more information on mechanisms of resistance and current levels of resistance.

Q: What controls do you recommend to ensure that the environment is anaerobic?

VH. At the reference laboratory we do not recommend biological controls such as a pseudomonas or positive growth of clostridium, because the control results are obtained the next day and that is too late; we use a chemical control. Oxoid sell Resazurine strips which go pink if anaerobic conditions are not achieved, so something can be done immediately. If using jars make sure they are maintained properly by changing the catalyst as necessary and ensuring that 'O' rings are cleaned and crack free.

Clinical data to support the interpretation of susceptibility of anaerobes

Speaker: Robin Howe

DL: How and why do you think *Bacteroides* is accumulating clindamycin resistance? I assume there is very little nosocomial transmission of *Bacteroides*. I assume that in the community you get a stable *Bacteroides* flora, not being replaced all the time. It can't survive very well on food (correct me if I'm wrong) and I assume that the average person is not exposed to frequent rounds of clindamycin in their life time. Why is this bug accumulating clindamycin resistance?

RH: I don't think we know that much about how much we share organisms. *Bacteroides* is not a particularly aero sensitive anaerobe. The data are from the US, (I don't have UK data), where clindamycin is used in the context of anaerobe infections.

DL: I would hope there weren't nosocomial infections with *Bacteroides*. I accept your point that it may survive better on food stuff and from faecal contamination generally than I would instinctively suppose. The average person would hardly ever come in to contact with clindamycin, more likely penicillin or erythromycin, so what is the drive there?

RH: I'm only suggesting that nosocomial infection could explain it, but I really don't know

Q: Could it be related to erythromycin use in UK?

RH: There is MLS_B type resistance and macrolide use could contribute to this.

Q: Are we likely to have the same pattern with *C. difficile* infection and metronidazole as with clindamycin?

RH: Metronidazole resistance is likely to increase along with its increased usage, but as yet resistance is very uncommon in *C. difficile*.

VH: Many clinical laboratories no longer isolate *C. difficile* but rely on toxin detection and therefore do not undertake susceptibility tests to metronidazole. This may be a hidden problem.

RH: From a clinical perspective, I think the best outcome is achieved by use of rapid EIA testing for toxin production followed by rapid institution of therapy. There is ongoing national surveillance to pick up metronidazole resistance as a significant problem in England. In addition local surveillance schemes could establish if resistance arises.

General questions and answers about BSAC methodology

Speaker: Jenny Andrews

Q. Will temocillin recommendations be in the next version on the website?

JA. Temocillin is currently being discussed by EUCAST with particular emphasis on the treatment of infections caused by ESBL and AmpC producing organisms. I am not sure if the company has addressed the issue of disc testing by BSAC methodology.

Q: In our laboratory we do a lot of breakpoint sensitivity testing and we are revising the way we test Staphylococci. We have a lot of MRSAs and it would be easier to set up a big battery of breakpoints and get them done in one go. Our clinical direction is that we require fusidic acid which has a BSAC MIC BP of 1 mg/L and I am trying to sort out a supply of antibiotic.

TA: We use breakpoints in the laboratory at Sheffield and test fucidin with Adatabs. We could let you know the supplier.

RH. Interestingly, using a trimethoprim MIC BP of 0.5 mg/L on the Phoenix system, we found that we were clipping the end of the 'wild susceptible' population and many of the staphylococci were resistant. After discussion at the Working Party, we have raised the trimethoprim MIC breakpoint to 1 mg/L so that many more isolates test susceptible.

Q. We use neomycin to treat organisms that are mupirocin resistant. What MIC BP should we use?

JA: There may be information on the 'wild susceptible' population on the SRGA web site and a BP might be suggested by this data.

RH: I am not aware of any correlation between resistance and efficacy with naseptin. Naseptin also contains chlorhexidine as well as neomycin and you are not testing the effect of chlorhexidine. You may want to review what the results are going to be used for.

Comment from the audience: I am sure the clinicians agree with you but they are looking at the resistance to mupirocin and what agents they can use.

Q: We test haemophilii by BSAC recommendations against a range of agents; we also have Moraxellae that we would ideally like to set up against the same range of antibiotics, but there are no zone diameters given for trimethoprim.

TW: Moraxellae are intrinsically resistant to trimethoprim and therefore there will be no recommendations.

Q: Can you give me a definition of chemotherapeutic index value? This is a question in the specialist portfolio.

RH: As far as I'm aware this is the difference between an antibiotic being active against an organism and its deleterious effects on man. The higher the chemotherapeutic index the less toxic the antibiotic would be.

DL: I have been asked by the Chairman to mention inducible Class A β -lactamases in a few species, *Proteus vulgaris*, *Proteus penneri* and *Citrobacter diversus*, that make susceptibility testing complicated. The big difference is that class A enzymes are inhibited by clavulanate so if we expose a *P. vulgaris* to ampicillin it is resistant but if we add clavulanic acid it becomes susceptible. Very occasionally *P. vulgaris*, *P. penneri* and *Citrobacter diversus* become derepressed for these Class A enzymes. In this case they tend to be resistant to cefotaxime and ceftriaxone but not to ceftazidime. Resistance still can be reversed by clavulanate. You might get caught into thinking the isolate is an ESBL producer. These are rarer pathogens and the inducible zone will vary and give resistance to ampicillin and susceptibility to co-amoxyclov. Derepression in these species is seen but rare.

Q: What about K1 enzymes in *Klebsiella oxytoca*?

DL: K1 is another Class A β -lactamase. It is chromosomal in *Klebsiella oxytoca* and it is not normally inducible. It is normally expressed at a low level, but occasionally there are variants that express it at a high level. If you see a *K. oxytoca* that is resistant to cefuroxime, piperacillin/tazobactam and aztreonam, if you happen to be testing it, that is susceptible to ceftazidime and very borderline to cefotaxime, then the odds are it's got a K1 enzyme. It can give a false positive on an ESBL test but tends to be weak. Cefpodoxime resistant and identification helps.

Q: If we are talking about methods to detect substantive resistance in Enterobacteriaceae and we had an organism that is cefpodoxime resistant, in terms of clinical management does it matter whether it has an AmpC or ESBL? Why should we differentiate the two?

DL: It does affect which antibiotics one can use. Fourth-generation cephalosporins, although not widely used in the UK, are an option against the AmpC producing organism. Against some of the ESBL producers the β -lactamase inhibitor combinations would remain options in mild to moderate infections. It is of some epidemiological importance that the big shift we see at present of ESBLs are of concern to every clinician and may be of use when forming antibiotic policies.

Q. About reporting of various organisms to HPA via CoSurv, only ESBL producers in Coliforms and Klebsiellae are currently required. Is it likely to change in the future and be extend to other coliforms?

DL. We accept the difficulty of recognising ESBL production in *Enterobacter* and *Citrobacter* species and whilst we are interested, we are sceptical about the quality of the data we are going to obtain in the short term and therefore we wouldn't want to pressure people on the issue at this time. There are bigger problems at this time and priorities to resolve. Compared with shifts to EUCAST based breakpoints which are going to increasingly introduce intermediate categories as well as sensitive and resistant.

Q. Relating to breakpoint testing, in particular for urine isolates and *Klebsiella* species with regard to co-amoxiclav and how we should report results?

DL. Report at face value for UTIs only not for serious infections.

Q. The BSAC template programme, would it be possible to add a mm scale to this so that zones could be measured?

TW. The new version is nearing completion. It should be fairly easy to put a scale across the middle; however the purpose of the template was to differentiate sensitive from resistant.

Comment from the audience. When doing QCs we measure up to 120 different zone sizes. We use the programme to produce templates with an intermediate category so that QCs fall in between two categories. We have found this an easy way of doing the QCs.

Q. If you are writing a template and enter a value for a sensitive zone diameter followed by a value for resistant, has anyone noticed it changes the sensitive value? If you put 19 mm in sensitive and 18 mm in resistant, it changes the sensitive value to 18mm.

TW. The next version won't do that but I will look at it.