

Standardized Disc Susceptibility Testing Method User Group Meeting

25 November 2003

Royal College of Physicians, London

QUESTIONS AND ANSWERS

Questions and Answers following Anna King's talk

Q: *The clinical outcome for the Stenotrophomonas in blood cultures, do you know how they correlated with the sensitive or resistance patterns?*

AK: No, the group did not correlate clinical response with susceptibility results.

C GK: I have a comment on the red dots at the right of the ceftaxime distribution graph. They have mecA genes and are the type of strains isolated in France and Germany. They are extremely heterogeneous and are very difficult to detect with any test but PCR.

Q: *Are they easier to detect at 35°C than 37°C?*

AK: No. NCCLS have decided to keep oxacillin/methicillin disc test instead of PCR and use ceftaxime as their confirmation test

JA: *A question for Gunnar. How has NCCLS tackled the problem of Stenotrophomonas?*

GK: They have not. NCCLS has committee meetings once a year and for the last 5 meetings no recommendations have been given.

Q: *Can Etests be used for Stenotrophomonas?*

AK: I have no idea if they can be used for testing *Stenotrophomonas*. Someone at the Brompton Hospital did MICs using Etest and zone sizes and got some peculiar results, however this was not repeated.

Stenotrophomonas is a slow growing organism so tests should be incubated for as long as possible before reading, preferably 24 h.

C: Your initial advice about not using Etest for testing *Stenotrophomonas* is sound advice. I have a suspicion that MICs are wrong from a therapeutic point of view.

Q: *We are currently using MIC BPs for our MRSA screening and methicillin is tested at 30° and 37°C. We are in the process of changing to ceftaxime, should we test at 30° or 37°C?*

TW: At the Hallamshire 37°C was used initially, but now we use 30°C that gives best separation using a concentration of 4 mg/L for testing.

Questions and Answers after Gunnar Kahlmeter's talk

Q: *What are the MIC distributions like for the cephalosporins that are more difficult to test?*

GK: The histograms for the 'wild sensitive' population are broader which confirms that they are more difficult to test particularly when measuring zone sizes of 35–45 mm for a 30 ug disc. *Stenotrophomonas* and *Enterobacter* give problems, but generally the distributions for *E. coli*, *Klebsiella pneumoniae*, Gram positives, *Citrobacter freundii* are acceptable.

Q: *What about second-generation cephalosporins that are near to the breakpoint?*

GK: Even with cephadroxil that is a less potent cephalosporin very much akin to cephalixin, distributions are as tight as those for gentamicin. The quality of distributions relies on good speciation.

Q: *Do you have any information on carbapenem distribution?*

GK: Yes and data is available on www.SRGA.org via the Internet.

If you go to zone diameter tables these are both Swedish (SRGA) and BSAC recommendations.

In most cases SRGA and the BSAC use the same disc content. Where there are differences generally SRGA discs are of a higher content, but SRGA is gradually changing to the lower BSAC disc contents.

Q: *Based on your observation would you argue that there is no point in doing sensitivity, you can just identify the organism and read your probability from the distribution and advise the clinicians on the probability of resistance?*

A: I understand what you are saying and to be quite honest you are right on many occasions and this is what clinicians do when they treat empirically. However, resistance cannot always be predicted and if zone or MIC data is collected it is always possible to re-evaluate or re-validate data at a later stage. Also measuring zones or MICs fits into the computer world which means that in my computer there are now data on just over 1 million *E. coli* which I can call serially, analyse, attach a patient age, whether community or intensive care unit strain etc.

Q: *What do you do with organisms like *Enterobacter cloacae* where many of the wild type strains will have inducible β -lactamases and you get zones to ampicillin and cephalosporins? Do you have interpretative rules?*

GK: A lot of those rules are 'no-no' rules. The odd chance that a β -lactam will be appropriate for therapy of an *Enterobacter cloacae* is so remote that it is better to report as resistant because testing is very difficult. So with *Enterobacter cloacae* all of the β -lactams are avoided and quinolones, aminoglycosides, cotrimoxazole are prescribed. I would be very careful in recommending any β -lactam therapy without a concomitant aminoglycoside.

Q JA: *Can I ask you a question about identifying organisms? I do not know whether it happens in Sweden but in England many laboratories report 'coliforms' as a cost saving exercise. How important do you think it is to identify organisms?*

GK: I think it is very important to identify organisms. I like to save money but 95% or more of Enterobacteriaceae can be identified by relatively cheap methods. With susceptibility testing the correct S or R relies on good identification.

Q JA: *How do people in the audience feel generally about the new idea from NEQAS where you are asked to speciate organisms?*

Vote: Majority like the idea, which is excellent news.

Q: *BSAC guidelines at the moment do not offer any zone sizes for topical antibiotics and I wonder whether there is anything on the Swedish website that would help?*

GK: No sorry. There is not going to be either as this is a hopeless area.

C JA: There is no organisation in the world looking at recommendations for topical antibiotics. All you can do is apply systemic interpretative criteria, but of course recommendations will not be available for everything for example kanamycin.

C: Pseudomonas in ears is the main area we use topical antibiotics for.

GK: I would do a recommended susceptibility test on these isolates knowing that I am probably overstating resistance. From a topical point of view I am giving the same type of information on important resistant mechanisms that I do for systemic problems and I am just as careful with identifying the MRSAs or the vancomycin resistant enterococci. From an epidemiological infection control point of view I do my best to do what I consider right. I realise that from a therapeutic point of view I might withhold an antibiotic that might actually be active because the resistance is at a lower level. If a bacterium has started down the path of resistance for example quinolone resistance then this group of antibiotics should be withheld.

General Discussion at end of the morning session

Q: *What are your views on automated sensitivity testing methods for example Vitek?*

AK: I do not have any difficulty with them, however you must realise that if you use the automated system that most of the expert rules are based on NCCLS and not on BSAC. The only thing I am concerned about is that users may accept the results without understanding the mechanisms of resistance. Having spoken to users they do inform me that they use the expert rules as an educational tool.

GK: I would have liked the manufacturers to give a broader range of MICs because the user would then know if an organism was within the wild type distribution. This added information helps the detection of resistance mechanisms. Currently we have to trust to whatever breakpoint is chosen by the manufacturer.

Q: *Over the past year we have seen a lot of multi resistant Acinetobacters in our hospital particularly on the ITU. What are your views on the reliability of sensitivity testing for Acinetobacter because some of the strains we have come out as resistant including the cephalosporins, carbapenems and we now do colistin and polymixin and we have actually used colistin on some of the patients? Can I believe those carbapenem results?*

AK: Carbapenems are not terribly active against Acinetobacter and there are some carbapenem resistant organisms being isolated. Some of these have enzymes that are recognised and some have a decrease in permeability but they are definitely resistant and they do exist in the UK.

C: Yesterday I had a patient on the ITU from whom we isolated an Acinetobacter that responded clinically to imipenem but on testing it was imipenem resistant.

AK: Imipenem is more active than meropenem against Acinetobacter so you would be better advised to use imipenem. If susceptibility testing shows that the isolate is resistant to imipenem then it will be equally resistant to meropenem.

GK: They are less difficult than Stenotrophomonas but a lot more difficult to test than other Gram negatives. The NCCLS have a working party just for Acinetobacter and they have decided to give up the β -lactams for the time being and concentrate on the rest of the drugs. They can get a test to work for all of the antibiotics, but cannot get a reasonable correlation between zones and MICs for the β -lactams.

As with Stenotrophomonas you may isolate an Acinetobacter from the patient, but this may not be the pathogen causing the infection. When a patient is treated with a carbapenem there is positive clinical response, the fever goes down, however a resistant Acinetobacter is isolated. This is a very difficult group of organisms to deal with.

Q: *The BSAC Enterobacteriaceae table has expert rules regarding Enterobacteriaceae about when to treat species differently. Is this what you are intending to do?*

GK: That is what EUCAST decided the other day.

Q: How does SRGA recommend detecting glycopeptide resistance in Staphylococci?

GK: We do not think this is a problem in Sweden because irrespective of which hospital you go to MRSA is less than 1% of the *S. aureus* population. Looking for vancomycin resistance of any kind would primarily be among the methicillin resistant isolates. To date we have had 147 MRSA this year in Sweden, we have full details on them, surname, age, gender etc. So we are not too worried about Vancomycin resistance at the moment. We are about to lose the battle in Stockholm and I would not be surprised to see it creeping into other areas. E-tests do not pick them up. There are two different resistance mechanisms, one heterogeneous low-level vancomycin resistance that we do not look for, all other *S. aureus* are screened for high-level resistance of the van type.

Q: *Is there any data for piperacillin/tazobactam against Stenotrophomonas?*

A: Dr. Robin Howe from Southmead Hospital in Bristol will be presenting clinical response data to the working party in February. It is hoped that this meeting will clarify the situation and we will report our findings on the web site or in a publication.

C TW: I know our clinicians use it and we have some sensitive zones for it on some of our strains.

C GK: I think there was a large analysis of clinical response data in *Stenotrophomonas* about 2 years ago and the conclusion was that there was a question mark for almost every antibiotic since it was difficult to establish if the *Stenotrophomonas* was causing the infection. It might be we have more information 2 years on we shall see.

AK: Dr Morrissey was responsible for identifying a true community acquired *S. maltophilia* pneumonia that a patient was admitted with. That patient was treated with ceftazidime and got better.

Q: *Question about quinolone testing. We are about to introduce moxifloxacin in our hospital, should we test this separately or extrapolate either nalidixic data or ciprofloxacin data?*

GK: Are you talking about Enterobacteriaceae?

A: Mostly, but pneumococcus as well.

GK: I certainly would test nalidixic acid and then extrapolate the moxifloxacin susceptibility.

AK: For instance, *E. coli* with a single mutation in a simple urinary tract infection will respond to ciprofloxacin. I think that if it is ciprofloxacin resistant then it would be moxifloxacin resistant.

GK: I do not want to give organisms with one or two mutations more ciprofloxacin or any other quinolone for that matter and clinicians should be dissuaded from prescribing quinolones for these isolates.

AK: I am sure that we have all noticed the high level of ciprofloxacin resistance that has increased exponentially in organisms that had a single mutation originally and have been treated with ciprofloxacin.

GK: You certainly get a lot of information if you do a nalidixic acid disc as a screen disc in conjunction with a ciprofloxacin disc test, and this can help in those cases where the clinician cannot use another alternative antibiotic.

AK: The one place where you might want to test moxifloxacin rather than ciprofloxacin is in pneumococci and chest infections. I think the BSAC recommendations make all pneumococci intermediate to ciprofloxacin. That is deliberate because ciprofloxacin, a less active quinolone, does not work well against pneumococci. It may therefore be worth testing moxifloxacin.

GK: The only two quinolones that would be allowed for pneumococci in the EUCAST harmonised table across Europe are moxifloxacin and levofloxacin. Moxifloxacin is the more active of the two but the pharmacokinetics of levofloxacin compensates for that. So on a wild type pneumococcus it is probably debatable which is the better one. There is good evidence to show that even the first mutation in an *S.*

pneumoniae will cause resistance to ciprofloxacin and probably to levofloxacin. Isolates might still be susceptible to moxifloxacin, but organisms with two mutations will be resistant to moxifloxacin. There is insufficient clinical data to know the response of all the quinolones when treating pneumococcal infections in the respiratory tree. To err on the side of caution EUCAST has dissuaded the use of ofloxacin and norfloxacin, but have allowed ciprofloxacin with intermediate as the most susceptible categorisation. When an organism acquires a first mutation it is no longer considered susceptible to ciprofloxacin or levofloxacin, but it will be considered susceptible to moxifloxacin. When an organism acquires two mutations it is considered resistant to moxifloxacin. In Europe, quinolone resistance in *S. pneumoniae* is less than 1% (EARRS programme) and that is good news.

AK: Gunnar, do you have any idea how much levofloxacin is used in treating pneumococci in Europe?

GK: In France heavily and the fighting match between the French and Germans is because levofloxacin is on the French side and Bayer is on the German side and they are both defending these drugs whereas norfloxacin is Astra Zeneca.

JA: Are some treatment failures because of under dosing in treating *H. influenzae* with levofloxacin?

GK: Jenny is right and I think the firm now realises and are doing studies to get the dose up.

Q: What do you put your low incidence of MRSA down to in Sweden?

GK: I wish we knew exactly because we could give some sound advice to others. I have a suspicion that we are on the tail of something that will happen in our country as in others. I think the fact that we are 8m people rather than 80m people in an area that is 3 or 4 times larger than the UK does help a lot. The fact that we have a socialistic system in our health care since 1910 actually helps; it is equal health care to everyone. We have been able to afford over the years single rooms for patients; therefore cross infection is less of a problem. We are still able to isolate every case with a suspected MRSA or where we have reason to suspect that there might be an MRSA but have not yet shown it. If someone comes in from Singapore they are put in isolation room for 3 days until it is known. A very conservative antibiotic policy, we are not able to get over the counter antibiotics, and a number of things like that add up together. We do not have one good answer for this.

AK: The MRSA is itself a strange organism. I remember when we first had it in this country, St Thomas's on the south side of the Thames never had it and people like UCH had many isolates and we had a joke that it would not cross the river. Now St Thomas's is one of the worst hospitals for MRSA.

GK: I think you have lost the battle when you cannot remember all the surnames of all your cases this year. The expense of containing it would be humungous and you would not convince your politicians to give you that money. The Gothenburg people had an epidemic with 357 cases over 3 year period and spent SK35 million to date to contain that epidemic (divide by 10 to get £). They managed to convince their politicians that it was worth it and managed to contain the epidemic and now there are no more cross infection cases. It is possible to contain at a low level but when it gets into the higher percentages then it is too late.

C: From an attendee from Guernsey in the Channel Isles.

We have a low incidence of MRSA. 10 years ago we did not have any, but we were not screening for it at that time, but we have picked it up relatively recently on routine swabs. Unfortunately we now have a high incidence but I think it is because people have to go to large hospitals on the main land such as Southampton and then bring it back. We are lucky because we are a private health care and can isolate patients especially in ITU. We have had to do a lot of learning.

Questions and Answers following David Livermore's talk

AK: *If you screen with cefpodoxime, can you infer cefuroxime sensitivity?*

DL: I do not think you can. You do get some cefuroxime resistance in *E. coli* due to minor reductions in permeability or up regulation of efflux. These strains have low level resistance to cefuroxime and ceftazidime but appear susceptible to the 3rd generation cephalosporins including cefpodoxime.

Q: *Would you use Piperacillin/tazobactam to treat an infection caused by an ESBL producer or would you always go with imipenem?*

DL: I would accept piperacillin/tazobactam results at face value. If an ESBL producer appears to be susceptible by disc testing I would count piperacillin/tazobactam as an acceptable therapy, though in the more seriously-ill patient I would prefer the carbapenem. If you start saying that you do not believe the susceptibility of ESBL producers to β -lactamase inhibitor combinations then you are saying you do not believe any result with a β -lactamase inhibitor combination. Why should you be cautious because it is an ESBL compared to a classical β -lactamase? Where the problem gets harder is where you are in the midst of an outbreak of ESBL producers and you have a patient who is sick and you want to give empirical therapy. You think it might be another ESBL producing strain and you know they are usually susceptible to piperacillin/tazobactam but you cannot be certain, since even within a single outbreak you get some representatives that are piperacillin/tazobactam susceptible and some that are resistant.

James Soothill: *We have had 2 Pseudomonas and one Klebsiella carrying carbapenemases and I regard that with considerable concern because with the aminoglycoside and quinolone resistance that is already going around, we are heading towards total resistance in some of the big hospitals in London and in the UK. I would be very grateful if anyone would be interested in setting up a screening system to try and find these things in faeces or other samples, because I am worried that some of the ones we found had MICs to meropenem and imipenem were just below the susceptible range. They are quite hard to find but I think we might have a silent spread going on before we find them*

DL: You and I have discussed this many times before. I agree metallo- β -lactamase producers are a growing concern and that they are difficult to detect. You can get false positives with the Etest MBL strips, but as you saw in the Taiwanese data and from your own experience as well, you also see strains that have MBL but which are not obviously carbapenem resistant. I wonder if the way to go might be to start looking for cephalosporin EDTA synergy rather than carbapenem EDTA synergy because BMLs more reliably give resistance to ceftazidime than they do to imipenem. It might increase the sensitivity of the test.

Q: *What do you think about combination of imipenem and a mercaptothial compound for ESBL detection?*

A: Unfortunately the thiol compound is very hazardous and is unacceptable to our staff safety.

AK: *Can I go back to the ESBLs and Klebsiella; why is piperacillin/tazobactam active against some strains but not others?*

DL: You can find answers in individual strains. Some have multiple different enzymes; some may have a permeability change; some just make a lot of one enzyme, what we cannot find is a real single global answer.

Q: *Automated systems such as the Vitek, will they detect all the enzymes you are talking about?*

DL: We tried the Vitek 2 and found that about 90% agreement to genetic testing for β -lactamases. That was OK using the card the manufacturer recommends, but I think problems arise where people customise the cards by, for example, having ceftazidime replaced by cephadrine, without realising how useful ceftazidime is to

interpretive reading. Once you start tinkering with the cards you start altering the ability to do good interpretive reading. Used properly they are a good answer but it is necessary to use them properly.

C: From a commercial point of view you can say our machine works better with our recommended card. But someone might buy the machine and want certain drugs put on the cards. What do we do? You cannot say "You are about to abuse the machine so I will not sell it to you." It gives a reasonable MIC.

AK: To be fair the manufacturers do say not to alter the cards. What about Vitek 1?

DL: I have less experience with this. There is a head to head comparison trial being run at Cambridge HPA at the moment between Vitek 1, 2 and Phoenix. Vitek 1 and Phoenix seem to identify resistance mechanisms based on specific tests eg for ESBLs, Vitek 2 best fits all the MICs to those for strains with known mechanisms to see which one the particular strain fits best. Other features of the machines are the cost, convenience etc. This is still to be evaluated.

Q: *About GP urines, should we introduce a cephalosporin screen for ESBLs or should we be sticking up the cephalexin and augmentin discs?*

DL: The best is to test cefpodoxime first line and to ESBL test those found resistant. If you have an ESBL producer it should be resistant to cephalexin and I have not seen one yet that is susceptible. But you will pick up other things that are cephalexin resistant, but are not ESBL producers, such as a strain that has got tons of a classical TEM enzyme or an Enterobacter or Citrobacter. You could argue, "I do not need to test cefpodoxime so what I will do is test cephalexin then I will pursue in some detail everything that comes up cephalexin-resistant to make sure if it is an ESBL producer." You could design a strategy along those lines. But I am not an enthusiast for cephalexin as therapy and would get shot of it and put cefpodoxime in instead. What do you think Gunnar?

GK: Yes that is what we do, test with cephalexin as first line screen and then put a lot of effort in to identify the rest. We look at BP to check for mechanisms of resistance but cephalexin resistance will not pick up cefuroxime resistance.

DL: Cefuroxime is one of the hardest drugs to test among regular agents used. The MICs and zones are close to the BPs, you get odd types of low-level permeability resistance.

GK: It is difficult to draw conclusions from anything else.

Q: *If you are using chromogenic agar with urines, is that sufficient for these guidelines?*

DL: If using chromogenic agar, you will know immediately if your strains is likely to be Enterobacter that is naturally resistant to cephalexin or an *E. coli* that may have an ESBL.

Q: *Because this does not split Enterobacter from Klebsiella does further identification to differentiate need to be done?*

DL: You would have to chase that further. Klebsiella is usually - not always - susceptible to cephalexin whereas Enterobacter is universally resistant. But the strategy does split out the *E. coli*.

Q: *We have been comparing piperacillin/tazobactam with ticarcillin/clavulanate and I was given to understand that they are interchangeable, but we are getting a small number of strains that are more resistant to ticarcillin/clavulanate. They are put on the same plates as the piperacillin/tazobactam; there are zones for piperacillin/tazobactam and there are no zones for ticarcillin/clavulanate.*

DL: Firstly, a higher concentration of tazobactam is used than clavulanate. Secondly, piperacillin is a weaker substrate than ticarcillin for lots of β -lactamases, so it tends to be easier to protect. So as long as isolates have ticarcillin/clavulanate resistance but are piperacillin/tazobactam susceptible then the result does not surprise me. If you start seeing the converse I will be surprised.

End of Day