

EXAMPLES OF NOTABLE ETEST® REFERENCES -2000

1. **A different approach to antimicrobial testing in cystic fibrosis.** Gallagher MJ*, Hart CA*, and MJ Walshaw.

Poster presentation, International Cystic Fibrosis Conference, Stockholm 2000.

“Standard techniques that determine bacterial antibiotic susceptibility assume that single bacterial strains cause acute infections, and may not be relevant in CF, where bacteria, especially *Ps. aeruginosa* (*Pa*), grow in biofilms and cause chronic colonisation. Furthermore, in CF multiple pathogens may be present, and one genotype of *Pa* may have many phenotypes. We have developed a new technique for antimicrobial sensitivity testing, where whole CF sputum is tested directly against Etest® strips (AB BIODISK) of Tobramycin, Meropenem, and Ceftazidime in turn. Following sputolysis, 1µl aliquots from 100 CF sputum samples were spread across agar plates by cotton wool swab, the Etest placed directly, and incubated for 2 days. Growth patterns allowed all bacterial MICs to be assessed, demonstrated interactions between all bacteria and antibiotics. In 99% of test cases a report was issued to the clinician, and traditional susceptibility methods were later compared. In 44% a halo of growth appeared at higher antibiotic levels, attributable to *Pa*, *B. cepacia* or *A. fumigatus* growing where other susceptible flora were inhibited. We believe this new method shows the complex polymicrobial ecology in CF and also helps to explain the differences seen between laboratory sensitivity testing and clinical response to antibiotic therapy.”

HIGHLIGHTS:

A ground breaking application for direct CF sputum testing with Etest that showed:

1. Bacterial interference, and how the presence of uninhibited normal flora in the CF lung e.g. viridans streptococci may inhibit the growth of a more dangerous CF pathogen like *P. aeruginosa*.
2. Different susceptibility results are seen for CF pathogens as they co-exist in polymicrobial populations.
3. Increased resistance of different CF bacterial phenotypes may be detected in the biofilm mode of growth that may be captured in the direct specimen testing method using Etest.
4. This new method may better mimic the *in vivo* ecology and provide newer perspectives on how treatment regimens need to be reassessed.

2. **Evaluation of the Etest method for determining voriconazole susceptibilities of 312 clinical isolates of *Candida* species by using three different agar media.** Pfaller MA, Messer SA, Houston A, Mills K, Bolmström A, Jones RN.

Journal of Clinical Microbiology, 2000 Oct; 38(10): 3715-7

Performance of the Etest for voriconazole susceptibility testing of 312 isolates of *Candida* spp. was assessed against that of the National Committee for Clinical Laboratory Standards (NCCLS) microdilution broth method. The NCCLS method employed RPMI 1640 broth medium, and MICs were read after incubation for 48 h at 35 degrees C. Etest MICs were determined with RPMI agar containing 2% glucose (RPG), Casitone agar (CAS), and antibiotic medium 3 (AM3) agar and were read after incubation for 48 h at 35 degrees C. The *Candida* spp. isolates included *C. albicans* (n = 174), *C. glabrata* (n = 55), *C. tropicalis* (n = 31), *C. parapsilosis* (n = 39), *C. krusei* (n = 5), *C. lusitaniae* (n = 2), and *C. guilliermondii* (n = 6). The Etest results obtained using RPG correlated well with the reference MICs. Overall agreement ranged from 91% for *C. glabrata* to 100% for *C. tropicalis*, *C. parapsilosis*, *C. guilliermondii*, *C. krusei*, and *C. lusitaniae*. When CAS was used, agreement ranged from 80% for *C. krusei* to 100% for *C. parapsilosis*, *C. guilliermondii*, and *C. lusitaniae*. With AM3, agreement ranged from 58% for *C. glabrata* to 100% for *C. lusitaniae* and *C. guilliermondii*. The Etest method using RPG appears to be a useful method for determining voriconazole susceptibilities of *Candida* species.

HIGHLIGHTS:

1. Etest method using RPMI agar provides reference quality MIC results for testing a new antifungal azole like voriconazole against the most important pathogens within *Candida* species.”
2. The Etest technique will be very attractive to laboratories because it provides the flexibility to test only one agent or a panel of antifungal agents representing different classes of systemic antifungal agents as the clinical situation dictates.

3. Evaluation of Etest for susceptibility testing of multidrug-resistant isolates of *Mycobacterium tuberculosis*. Hazbon MH, del Socorro Orozco M, Labrada LA, Tovar R, Weigle KA, Wanger A.

Journal of Clinical Microbiology, 2000 Dec; 38(12):4599-603.

To prescribe effective treatment schemes for patients with tuberculosis, more-efficient susceptibility testing techniques for *Mycobacterium tuberculosis* are needed, especially in regions with multidrug resistance. Etest (AB BIODISK, Solna, Sweden) is a simple technique that provides quantitative drug susceptibility results for *M. tuberculosis* in 5 to 10 days from a culture grown at low cost. The performance of Etest was compared to that of the reference proportion method, using 95 *M. tuberculosis* clinical isolates of which 42.1% (40 of 95) were resistant to at least one antibiotic by the reference method. Overall agreement between Etest and the reference method was 98.9% (94 of 95) for detection of multidrug resistance; for resistance to individual drugs, agreement was 97.9% (93 of 95) for rifampin, 96.0% (92 of 95) for ethambutol, 94.7% (90 of 95) for isoniazid, and 85.3% (81 of 95) for streptomycin. This study supports the utility of Etest for timely detection of drug resistance in *M. tuberculosis* and for use in tuberculosis control programs.

HIGHLIGHTS:

1. The positive and negative predictive values of Etest indicate that the technique is able to accurately detect resistance and susceptibility of *M. tuberculosis* to the four agents and their combinations.
2. Etest can positively impact TB control as a tool for individual patient testing and surveillance programs in settings with high resistance prevalences.
3. Etest advantages for the TB application include shorter turnaround time, simplicity in performance, and lower cost.

EXAMPLES OF NOTABLE ETEST[®] REFERENCES -2001

1. Evaluation of current methods for detection of staphylococci with reduced susceptibility to glycopeptides. Walsh TR, Bolmström A, Qwarnstrom A, Ho P, Wootton M, Howe RA, MacGowan AP, Diekema D.

Journal of Clinical Microbiology, 2001 July; 39(7): 2439-44.

The sensitivity and specificity of seven methods (agar dilution, broth microdilution, Etest at 0.5 and 2.0 McFarland (McF) inocula, two agar screening methods, and population studies [PS]) were evaluated in a double-blind study involving 284 methicillin-resistant *Staphylococcus aureus* (MRSA) strains and 45 *Staphylococcus* strains with reduced susceptibilities to vancomycin (SRSV). The results were compared to the population analysis profile-area under the curve ratio method (PAP-AUC ratio compared to that of Mu3) as described by Wootton *et al.* The agar screening method using brain heart infusion agar (6 microg of vancomycin per ml) gave a sensitivity of 22% and a specificity of 97%. A similar method using Mueller-Hinton agar (5 microg of vancomycin per ml) gave a sensitivity of 20% and a specificity of 99%. The PS method detected 34 false positives (12%) and gave a sensitivity of 71% and a specificity of 88%. Etest using 0.5 and 2.0 McF inocula gave sensitivities and specificities of 82 and 93% and of 96 and 97%, respectively. The best Etest interpretative criteria for the 2.0 McF inoculum was ≥ 8 mg of vancomycin per liter and ≥ 8 microg teicoplanin per ml or ≥ 12 microg of teicoplanin per ml. The direct colony suspension inoculum for this method was found to be equally accurate in detecting (hetero-) glycopeptide-intermediate *S. aureus* compared to the overnight broth inoculum preparation method. Agar dilution and broth

microdilution using the NCCLS breakpoint criteria for vancomycin gave sensitivities and specificities of 20 and 100% and of 11 and 100%, respectively. Using the Etest with a 2.0 McF inoculum, six different media were assessed against a selection of SRSV (n = 48) and MRSA (n = 12). Brain heart infusion agar yielded the highest sensitivity and specificity values: 88 and 88%, respectively.

HIGHLIGHTS:

1. Etest, in contrast to reference methods, could be effectively adapted to a higher inoculum and a richer medium such as BHI to optimize the detection of hGISA.
2. The use of the Etest under the macromethod conditions provides a reliable and sensitive method for the detection of subtle variations in glycopeptides resistance, including heteroresistance.

2. Heteroresistance to vancomycin in *Enterococcus faecium*. Alam MR, Donabedian S, Brown W, Gordon J, Chow JW, Zervos MJ, Hershberger E.

Journal of Clinical Microbiology, 2001 September; 39(9): 3379-81.

This study presents the first report of vancomycin heteroresistance in an *Enterococcus faecium* isolate from a patient. The original isolate was susceptible *in vitro* to vancomycin. Etests showed growth of subcolonies in a zone of inhibition with a vancomycin MIC of >256 microg/ml. Both the susceptible and resistant colonies were from the same strain as determined by PFGE, and both contained the vanA gene as determined by PCR.

HIGHLIGHTS:

1. This is a first report of a hetero-vancomycin-resistant *E. faecium* (hetero-VSEF) isolate; importantly it was isolated from a patient with endocarditis.
2. This isolate was reported to be susceptible to vancomycin *in vitro*, however, Etest results showed a subpopulation of isolates resistant to vancomycin.
3. Automated testing methods may not detect the presence of resistant subpopulations and a subsequent adverse outcome may occur when an inappropriate use of vancomycin is combined with testing methods based on broth microdilution, especially if a rapid reading of results is performed.
4. The heterogeneity of the VSEF isolate was observed only by Etest, and the automated Microscan method was not adequate for detecting the heteroresistance.

3. Determination of MICs in the routine laboratory. Shah PM.

Journal of Antimicrobial Chemotherapy, Correspondence, 2001 December; 48(6): 931.

“In the age of automation and commercially available microtitre plates for MIC determination, more and more laboratories use these methods, although the report of the Working Party on Susceptibility Testing of the BSAC clearly shows that in the routine laboratory, an agar diffusion technique, if performed correctly, delivers results satisfactory for patient care.¹ Post-commercially available MIC microtitre plates lack concentrations at the lower end of the panel. As a result, low MICs are reported as ‘≤ xyz mg/L’, thus the ‘true’ MIC is often not determined. I randomly selected a recent publication from the Journal. Johnson *et al.*² report a MIC range of ≤ 0.06–2 and MIC₅₀ and MIC₉₀ of ≤ 0.06 mg/L of penicillin for surveillance isolates of pneumococci from England and Wales. The MIC of penicillin was not determined for at least 90% of the strains.

When using commercially available MIC plates, one ends up working with an extended ‘breakpoint’ method, as the number of dilutions is generally limited. Routine methods should be designed to detect slight but clinically significant shifts in susceptibility patterns (see gradual shift in susceptibility to penicillin in *Neisseria gonorrhoeae*,³ or more recently in *Streptococcus pneumoniae*,⁴ or to ciprofloxacin in *Salmonella*⁵). If MIC determination is used, concentrations chosen should allow end-point determination. To my knowledge, PROTEKT (Prospective Resistance Organism Tracking and Epidemiology for the Ketolide Telithromycin)

has initiated a programme to do just this, albeit for limited species of bacterial pathogen. At present, Etest is the one (if not only) commercially available test method that allows 'true' determination of MIC."

HIGHLIGHTS:

1. Commercial broth microdilution products have limited dilutions and are essentially extended breakpoint methods.
2. Off-scale results i.e. \leq or \geq are not MIC values and are not useful for surveillance purposes for detecting subtle shifts in susceptibility levels.
3. ***"At present, Etest is the one (if not only) commercially available test method that allows 'true' determination of MIC."***

4. Evaluation of Etest method for determining caspofungin (MK-0991) susceptibilities of 726 clinical isolates of *Candida* species. Pfaller MA, Messer SA, Mills K, Bolmstrom A, Jones RN.

Journal of Clinical Microbiology, 2001 Dec; 39(12): 4387-9.

The performance of the Etest for testing the susceptibilities to caspofungin (MK-0991) of 726 isolates of *Candida* spp. was assessed against the National Committee for Clinical Laboratory Standards (NCCLS) microdilution broth method. The NCCLS method employed RPMI 1640 broth medium, and MICs were read after incubation for 48 h at 35 degrees C. MICs were determined by Etest for all 726 isolates with RPMI agar containing 2% glucose (RPG) and were read after incubation for 48 h at 35 degrees C. The *Candida* isolates included *Candida albicans* (n = 486), *Candida glabrata* (n = 96), *Candida tropicalis* (n = 51), *Candida parapsilosis* (n = 47), *Candida krusei* (n = 11), *Candida lusitanae* (n = 2), and *Candida guilliermondii* (n = 33). In addition, a subset of 314 isolates was also tested by Etest using Casitone agar (CAS) and antibiotic medium 3 agar (AM3). The Etest results obtained using RPG correlated well with reference MICs. Overall agreement was 94% with RPG, 82% with CAS, and 79% with AM3. When RPG was used, agreement ranged from 79% for *C. parapsilosis* to 100% for *C. krusei*, *C. lusitanae*, and *C. guilliermondii*. When CAS was used, agreement ranged from 0% for *C. lusitanae* to 100% for *C. glabrata*. With AM3, agreement ranged from 0% for *C. lusitanae* to 100% for *C. guilliermondii*. All three media supported growth of each of the *Candida* species. Etest results were easy to read, with sharp zones of inhibition. In most instances (75%) where a discrepancy was observed between the Etest and the reference method, the Etest MIC was lower. The Etest method using RPG appears to be useful for determining caspofungin susceptibilities of *Candida* species.

HIGHLIGHTS:

1. Etest using RPMI agar can be used to test *Candida*, *Cryptococcus*, and other yeasts against polyenes, triazoles, and new and unique antifungal agents such as glucan synthesis inhibitors e.g. caspofungin.
2. Etest offers great flexibility to laboratories that may want to test one or two agents yet provide quantitative MIC data that are comparable to reference broth dilution results.
3. The flexibility of the Etest technology will stimulate additional unique applications that may add to the understanding of the *in vitro* interactions between fungi and antifungal agents.

5. Improved detection of amphotericin B-resistant isolates of *Candida lusitanae* by Etest. Peyron F, Favel A, Michel-Nguyen A, Gilly M, Regli P, Bolmström A.

Journal of Clinical Microbiology, 2001 Jan; 39(1): 339-42.

Both intrinsic and acquired resistance to amphotericin B has been documented for *Candida lusitanae*. Amphotericin B remains the drug of choice for many critical fungal infections, and the detection of resistance is essential to monitor treatment effectively. The limitations of the National Committee for Clinical Laboratory Standards (NCCLS) reference methodology for detection of amphotericin B resistance

are well documented, and several alternative methods have been proposed. Etest assays with RPMI and antibiotic medium 3 (AM3) agar were compared to the NCCLS M27-A broth macrodilution method using AM3 for amphotericin B resistance testing with 49 clinical isolates of *C. lusitaniae*. The panel included nine isolates with known or presumed resistance to amphotericin B on the basis of *in vivo* and/or *in vitro* data. The distribution of amphotericin B MICs by Etest with RPMI ranged from 0.032 to 16 microg/ml and was bimodal. All of the putatively resistant isolates were inhibited by amphotericin B at ≥ 0.38 microg/ml and could be categorized as resistant using this breakpoint. Etest with AM3 yielded a broader amphotericin B MIC range (0.047 to 32 microg/ml), and there were six putatively resistant isolates for which MICs were >1 microg/ml. The separation of putatively susceptible and resistant isolates was less obvious. Broth macrodilution with AM3 generated a unimodal distribution of MICs (ranging from 0.032 to 2 microg/ml) and failed to discriminate most of the putatively resistant isolates at both 24 and 48 h. Etest using RPMI and, to a lesser extent, using AM3 provided better discrimination between amphotericin B-resistant and -susceptible isolates of *C. lusitaniae*.

HIGHLIGHTS:

1. The need for a reliable and clinically relevant method for amphotericin B susceptibility testing of yeasts is important since the reference method remains inefficient for resistance detection.
2. Previous studies have demonstrated that Etest provides more clinically significant amphotericin B results than did the standard methodology.
3. This study provides additional evidence of the superiority of Etest over the reference NCCLS broth dilution method for detection of resistance to amphotericin B among isolates of *C. lusitaniae*.

6. Evaluation of Etest for direct antifungal susceptibility testing of yeasts in positive blood cultures. Chang HC, Chang JJ, Chan SH, Huang AH, Wu TL, Lin MC, and TC Chang.

Journal of Clinical Microbiology, April 2001, Vol 39, No. 4, p 1328-1333.

The performance of the Etest (AB BIODISK, Solna, Sweden) for direct antifungal susceptibility testing of yeasts in positive blood cultures was compared with that of the macrodilution method for determining the MICs of five antifungal agents. Culture broths with blood from bottles positive for yeasts were inoculated directly onto plates for susceptibility testing with the Etest, and the MICs were read after 24 and 48 h of incubation. A total of 141 positive blood cultures (72 cultures of *Candida albicans*, 31 of *Candida tropicalis*, 14 of *Candida glabrata*, 11 of *Candida parapsilosis*, 3 of *Candida krusei*, and 3 of *Cryptococcus neoformans*, 4 miscellaneous yeast species, and 3 mixed cultures) were tested, and the rates of MIC agreement (± 1 log(2) dilution) between the direct Etest (at 24 and 48 h, respectively) and macrodilution methods were as follows: amphotericin B, 81.8 and 93.5%; flucytosine, 84.8 and 87.7%; fluconazole, 89.4 and 85.5%; itraconazole, 69.7 and 63.8%; ketoconazole, 87.9 and 79.0%. By a large-sample t test, the difference in log(2) dilution between the direct Etest and the macrodilution method was found to be small ($P < 0.05$). The lone exceptions were ketoconazole at 48 h of incubation and itraconazole at both 24 and 48 h of incubation ($P > 0.05$). By Tukey's multiple comparisons, the difference between the direct Etest (48 h) and reference methods among different species was found to be less than 1 log(2) dilution. When the MICs were translated into interpretive susceptibility, the minor errors caused by the direct Etest (at 24 and 48 h, respectively) were as follows: flucytosine, 2.3 and 1.4%; fluconazole, 3.0 and 3.6%; itraconazole, 21.2 and 21.3%. Itraconazole also produced an additional 3.0 and 3.6% major errors as determined by the direct Etest at 24 and 48 h, respectively. It was concluded that, except for itraconazole, the Etest method was feasible for direct susceptibility testing of blood cultures positive for yeasts. The method is simple, and the results could be read between 24 and 48 h after direct inoculation, whenever the inhibition zones were discernible.

HIGHLIGHTS:

1. Direct susceptibility testing of positive blood cultures with Etest showed a good correlation with the reference NCCLS method.

2. The direct Etest method is simple and less labor-intensive and the MIC results are available within 24 to 48 h after a positive blood culture containing yeast is found.
3. The direct Etest method can save up to 2 days compared to the standard procedures encompassing strain isolation followed by susceptibility testing which is a significant advantage for patient care.

EXAMPLES OF NOTABLE ETEST[®] REFERENCES -2002

1. Comparison of Etest, chequerboard dilution and time-kill studies for the detection of synergy or antagonism between antifungal agents tested against *Candida* species. Lewis RE, Diekema DJ, Messer SA, Pfaller MA, Klepser ME.

Journal of Antimicrobial Chemotherapy, 2002 Feb;49(2):345-51.

Currently, there is considerable debate regarding the best in vitro method for testing antifungal combinations against *Candida* spp. In this study, we compared the results obtained by chequerboard dilution, time-kill studies and Etest for several antifungal combinations against *Candida* spp. Three *Candida albicans* isolates (fluconazole MICs of 1.0, 32 and >256 mg/L) and three non-*albicans Candida* isolates (*C. glabrata*, *C. tropicalis* and *C. krusei*) were tested in RPMI 1640 medium. By chequerboard testing, the majority of antifungal combinations were found to be indifferent. Notably, antagonism was identified by time-kill studies and by Etest for combinations of amphotericin B-fluconazole, but it was not detected by the chequerboard method. Pre-exposure of isolates to fluconazole did not affect results of the Etest or chequerboard method, but it did increase the frequency of antagonism noted by time-kill methods. This study indicates that chequerboard dilution testing in RPMI medium may not reliably detect the attenuation of amphotericin B activity. Of the three methods, Etest was the simplest to use and yielded reproducible results for testing antifungal combinations.

HIGHLIGHTS:

1. Etest results for combination testing of antifungal agents could be an acceptable alternative to reference time-kill studies.
2. The Etest techniques employed for antifungal combination testing is simple to use, time-efficient and can give reproducible results.

2. Evaluation of a new Etest for detecting metallo-beta-lactamases in routine clinical testing. Walsh TR, Bolmstrom A, Qvarnstrom A, Gales A.

Journal of Clinical Microbiology. 2002 Aug;40(8):2755-9.

Several Etest (AB BIODISK, Solna, Sweden) gradient formats were developed for detection of metallo-beta-lactamases based on the reduction of imipenem (IP) or ceftazidime (TZ) MICs in the presence of EDTA or 2-mercaptopyruvic acid (MPA). The Etest metallo-beta-lactamase (Etest MBL) strips consisted of a double-sided seven-dilution range of IP or TZ (4 to 256 microg/ml) and IP or TZ (1 to 64 microg/ml) overlaid with a constant concentration of EDTA or MPA. The prototype strips were evaluated on several agar media (brain heart infusion agar, Isosensitest agar, nutrient agar, and Mueller-Hinton agar for aerobes and brucella blood agar for anaerobes) with 138 challenge strains: *Acinetobacter* spp. (n = 9), *Aeromonas* spp. (n = 8), *Chryseobacterium* spp. (n = 28), *Escherichia coli* (n = 1), *Klebsiella pneumoniae* (n = 4), *Pseudomonas aeruginosa* (n = 14), *Proteus mirabilis* (n = 3), *Serratia* spp. (n = 10), *Stenotrophomonas maltophilia* (n = 43), *Sphingobacterium* spp. (n = 3), and *Bacteroides fragilis* group (n = 15). PCR analysis using specific primers for IMP-1, L1, CcrA, and bla(B/C) confirmed the presence of the metallo-beta-lactamase genes. Enzyme assays were also performed with IP as an indicator substrate followed by EDTA inhibition profiles. EDTA was found to be a better inhibitor of metallo-beta-lactamases, especially for anaerobes. IP was a better than TZ. Mueller-Hinton agar was the preferred medium, particularly when compared to Isosensitest agar, which frequently produced falsely low MICs for IP. Etest IP plus IP-EDTA with Mueller-Hinton agar had a sensitivity of 94% (79 of 84) and specificity of 95% (124 of 130). The Etest MBL strip appears to be an

acceptable diagnostic reagent to detect metallo-beta-lactamase phenotypes in the clinical microbiology laboratory.

HIGHLIGHTS:

1. The new Etest MBL strip has the ability to detect metallo- β -lactamases (MBL), both chromosomally and plasmid mediated, in aerobic and anaerobic bacteria.
2. The novel Etest method can be used by clinical laboratories to monitor the emergence of MBL among clinically significant bacteria and for surveillance networks to study the spread of the enzyme.

EXAMPLES OF NOTABLE ETEST[®] REFERENCES -2003

1. **Evaluation of Etest method for determining fluconazole and voriconazole MICs for 279 clinical isolates of *Candida* species infrequently isolated from blood.** Maxwell MJ, Messer SA, Hollis RJ, Boyken L, Tendolkar S, Diekema DJ, Pfaller MA. *Journal of Clinical Microbiology*, 2003 March; 41(3): 1087-90.

The performance of Etest in fluconazole and voriconazole testing of 279 isolates of uncommon *Candida* spp. was assessed in comparison with the National Committee for Clinical Laboratory Standards (NCCLS)-approved standard broth microdilution (BMD) method. The NCCLS method employed RPMI 1640 broth medium, and MICs were read after incubation for 48 h at 35 degrees C. Etest MICs were determined with RPMI agar containing 2% glucose and were read after incubation for 48 h at 35 degrees C. The isolates include *Candida krusei*, *C. lusitanae*, *C. guilliermondii*, *C. kefyr*, *C. rugosa*, *C. lipolytica*, *C. pelliculosa*, *C. dubliniensis*, *C. famata*, *C. zeylanoides*, *C. inconspicua*, and *C. norvegensis*. Overall agreement between Etest and BMD MICs was 96% for fluconazole and 95% for voriconazole. Where a discrepancy was observed between Etest and the reference method, the Etest tended to give lower values with both fluconazole and voriconazole. The Etest method using RPMI agar appears to be a useful method for determining fluconazole and voriconazole susceptibilities of uncommon species of *Candida*.

HIGHLIGHTS:

1. The study confirmed those of previous reports regarding the ability of Etest to generate reliable fluconazole and voriconazole MIC data for the less common species of *Candida*.
2. Etest with RPMI agar can be used to determine reference quality MICs of fluconazole and voriconazole against rare and common species of *Candida* causing candidemia.
3. Several of these species although uncommon causes of BSI, may exhibit innate or acquired resistance to both amphotericin B and fluconazole and "reference quality" antifungal testing capabilities as shown by Etest can aid management decisions.

2. **Geographical difference in antimicrobial resistance pattern of *Helicobacter pylori* clinical isolates from Indian patients: Multicentric study.** Thyagarajan SP, Ray P, Das BK, Ayyagari A, Khan AA, Dharmalingam S, Rao UA, Rajasambandam P, Ramathilagam B, Bhasin D, Sharma MP, Naik SR, Habibullah CM. *Journal of Gastroenterology and Hepatology*, (2003) 18, 1373–1378.

Abstract

Aim: To assess the pattern of antimicrobial resistance of *Helicobacter pylori* isolates from peptic ulcer disease patients of Chandigarh, Delhi, Lucknow, Hyderabad and Chennai in India, and to recommend an updated anti-*H. pylori* treatment regimen to be used in these areas.

Methods: Two hundred and fifty-nine *H. pylori* isolates from patients with peptic ulcer disease reporting for clinical management to the Post Graduate Institute of Medical Education and Research, Chandigarh; All India Institute of Medical Sciences, New Delhi; Sanjay Gandhi Post Graduate Institute of Medical Sciences,

Lucknow; Deccan College of Medical Sciences and Allied Hospitals, Hyderabad; and hospitals in Chennai in collaboration with the Dr ALM Post Graduate Institute of Basic Medical Sciences were analyzed for their levels of antibiotic susceptibility to metronidazole, clarithromycin, amoxicillin, ciprofloxacin and tetracycline. The Etest, a quantitative antibiotic susceptibility testing method, was adopted in all the centers. The pattern of single and multiple resistances at the respective centers and at the national level were analyzed.

Results: Overall *H. pylori* resistance rate was 77.9% to metronidazole, 44.7% to clarithromycin and 32.8% to amoxicillin. Multiple resistance was seen in 112/259 isolates (43.2%) and these were two/three and four drug resistance pattern to metronidazole, clarithromycin, amoxicillin observed (13.2, 32 and 2.56%, respectively). Metronidazole resistance was high in Lucknow, Chennai and Hyderabad (68, 88.2 and 100%, respectively) and moderate in Delhi (37.5%) and Chandigarh (38.2%). Ciprofloxacin and tetracycline resistance was the least, ranging from 1.0 to 4%.

Conclusion: In the Indian population, the prevalence of resistance among *H. pylori* is very high to metronidazole, moderate to clarithromycin and amoxicillin and low to ciprofloxacin and tetracycline. The rate of resistance was higher in southern India than in northern India. The Etest emerges as a reliable quantitative antibiotic susceptibility test. A change in antibiotic policy to provide scope for rotation of antibiotics in the treatment of *H. pylori* in India is a public health emergency.

HIGHLIGHTS:

1. The use of Etest for a challenging fastidious organism such as *H. pylori* that requires significant experience was successfully achieved in a multi-site study in India.
2. Important national and regional epidemiologic resistance patterns against commonly used drugs for *H. pylori* treatment could be generated using Etest.
3. Etest can aid the changes in antibiotic policy to provide scope for rotation in treatment of *H. pylori* in India which comprise a public health emergency.

3. Use of the Etest to assess synergy of antibiotic combinations against isolates of *Burkholderia cepacia*-complex from patients with cystic fibrosis. Manno G, Ugolotti E, Belli ML, Fenu ML, Romano L, Cruciani M.

European Journal of Clinical Microbiology & Infectious Diseases, (2003), 22(1):28-34.

Treatment of *Burkholderia cepacia*-complex infections in cystic fibrosis patients is problematic, since the microorganism is often resistant to most antimicrobial agents. In this study, the Epsilon meter test, or Etest, was used to assess the activity of antimicrobial combinations against *Burkholderia cepacia*-complex. In a preliminary evaluation, the Etest was compared to the checkerboard method using 10 test organisms. Synergy testing by the Etest was then performed on 131 clinical isolates of *Burkholderia cepacia*-complex using various combinations of antimicrobial agents. Agreement between the Etest and the checkerboard method was 90%. The rate of resistance to individual agents ranged from 48% for meropenem to 100% for tobramycin, chloramphenicol, and rifampin. In 71.6%, 15.6%, and 12.6% of the test evaluations performed, the combinations tested resulted in additivity/indifference, synergism, and antagonism, respectively. The highest rates of synergy were observed with combinations of ciprofloxacin-piperacillin (44%), rifampin-ceftazidime (33%), chloramphenicol-ceftazidime (22%), cotrimoxazole-piperacillin/tazobactam (22%), and ciprofloxacin-ceftazidime (21%). Rates of antagonism for cotrimoxazole and chloramphenicol in combination with beta-lactam agents were higher than those observed for ciprofloxacin plus beta-lactam agents. These results suggest that the Etest is a valuable and practical method to be considered for improving the identification of possible therapeutic options in cystic fibrosis patients infected with organisms belonging to the *Burkholderia cepacia*-complex.

HIGHLIGHTS:

1. The Etest method allowed for the rapid screen of the combined activity of multiple combinations of antibiotics against isolates of *Burkholderia cepacia*.
2. The modified Etest technique for synergy studies is simple to use, time efficient and reproducible and is now increasingly being used.

3. This study support further evaluation of Etest for improving therapeutic options for pulmonary exacerbations of cystic fibrosis associated with *Burkholderia cepacia* and other microorganisms.

EXAMPLES OF NOTABLE ETEST[®] REFERENCES -2004

1. **Pharmacokinetic/pharmacodynamic modeling can help guide targeted antimicrobial therapy for nosocomial gram-negative infections in critically ill patients.** Mohr JF, Wanger A, Rex JH.

Diagnostic Microbiology and Infectious Disease, 2004 February; 48(2): 125-30.

Critically ill patients have altered pharmacokinetics (PK) that needs to be considered when choosing and dosing antibiotics. We conducted a prospective, observational study to assess clinical and microbiologic response rates in 19 critically ill patients with nosocomial Gram-negative infections. Antibiotics were dosed based on a mathematical pharmacodynamic (PD) model accounting for these altered kinetic parameters. The average APACHE II score +/- SE on intensive care unit admission and at the time of infection was 13.6 +/- 1.2 and 14.6 +/- 1.1, respectively. With targeted antimicrobial therapy adjusted to achieve an optimal PD profile, 17/19 (89%) patients had a clinical cure or improvement and 16/19 (84%) had either microbiologic eradication or presumed eradication. Modeling PD in these critically ill patients resulted in good clinical and microbiologic outcomes.

HIGHLIGHTS:

1. Etest was used to generate the exact MIC values for pharmacodynamic modeling to help target antibiotic therapy of serious Gram negative nosocomial infections in critically ill patients
2. Based on the MIC-PK/PD models, the most potent agent within the class could be utilised and this would increase the probability of achieving the PK/PD target while eliminating kinetic variability.
3. Optimization of antimicrobial therapy based on MIC-PK/PD modelling was well received by the primary physicians.
4. High rates of clinical cure and microbiologic eradication was achieved using MIC targeted therapy.

2. **Vancomycin-resistant *Staphylococcus aureus* - New York, 2004.** Kacica M, McDonald LC. *Morbidity and Mortality Weekly Report*, April 23, 2004; 53(15): 322-323.

Staphylococcus aureus is a common cause of hospital- and community-acquired infections (1,2). The development of vancomycin-resistant enterococci in 1988 led the way to the emergence of vancomycin-resistant *S. aureus* (VRSA) (minimum inhibitory concentration [MIC] [greater than or equal to] 32 [micro]g/mL [3]), first recognized in 2002 (4-7). This report describes the third documented clinical isolate of VRSA from a patient in the United States and provides evidence of failure to detect this VRSA by commonly used automated antimicrobial susceptibility testing.

HIGHLIGHTS:

1. The isolate tested using Microscan[®] overnight panels gave a vancomycin result of MIC 4 µg/mL while Etest showed the isolate to be highly resistant to vancomycin (MIC >256 µg/mL).
2. After notification and subsequent analysis by the New York State Department of Health (NYSDOH), the isolate was forwarded to CDC where it was confirmed to be VRSA (vancomycin resistant *S. aureus*); MIC = 64 µg/mL.

2. Although the VRSA isolate contained the *vanA* resistance gene, the vancomycin MIC appeared low when tested initially by an automated method.
3. Additional testing at CDC indicated that Microscan® and Vitek® testing panels and cards available in the United States did not detect vancomycin resistance in this VRSA isolate.
4. Consequently, it may be possible that additional VRSA infections might have occurred but were undetected by laboratories using automated methods.
5. Potential VRSA isolates should be saved for confirmatory testing, and clinical microbiology laboratories must ensure that they are using susceptibility testing methods that will detect VRSA.
6. “The most accurate form of vancomycin susceptibility testing for staphylococci is a non automated MIC method (e.g., broth microdilution, agar dilution, or agar-gradient (Etest) diffusion) in which the organisms are incubated for a full 24 hours before reading results.”

EXAMPLES OF NOTABLE ETEST® REFERENCES -2005

1. **Breakpoints for predicting *Pseudomonas aeruginosa* susceptibility to inhaled tobramycin in cystic fibrosis patients: use of high-range Etest strips.** Morosini MI, Garcia-Castillo M, Loza E, Perez-Vazquez M, Baquero F, Canton R.

Journal of Clinical Microbiology, 2005 Sep;43(9):4480-5.

Inhaled administration of tobramycin assures high concentrations in cystic fibrotic lungs, improving the therapeutic ratio over that of parenteral tobramycin levels, particularly against *Pseudomonas aeruginosa*. Conventional Clinical and Laboratory Standards Institute (CLSI; formerly National Committee for Clinical Laboratory Standards) breakpoints only consider parenteral levels and do not take into account these high antimicrobial concentrations. The Spanish Antibiogram Committee (The MENSURA Group) has tentatively defined specific breakpoint values for inhaled tobramycin when testing *P. aeruginosa* isolates from cystic fibrosis (CF) patients (susceptible, < or =64 microg/ml; resistant, > or =128 microg/ml). The antimicrobial susceptibilities of 206 prospectively collected CF *P. aeruginosa* isolates were determined by the reference agar dilution method. For tobramycin, the performance of high range tobramycin Etest strips (AB Biodisk, Solna, Sweden) and conventional tobramycin disks were assessed with the same collection. Applying MENSURA proposed breakpoints, 95.1% of the strains were categorized as susceptible to tobramycin, either using agar dilution or Etest high-range strips (99% categorical agreement between both methods). With CLSI breakpoints, susceptibility rates decreased to 79.1 and 81.1% for agar dilution and Etest strips, respectively (83.5% categorical agreement). Minor, major, and very major errors for Etest strips (CLSI criteria) were 13.6, 1.2, and 14.8%, respectively. Upon applying the new proposed criteria for inhaled tobramycin, only one major and one very major error were observed with Etest strips. Whenever inhaled tobramycin is considered for therapy, we suggest that *P. aeruginosa* strains from CF patients categorized as intermediate or resistant to tobramycin according to the CLSI criteria should be retested with high-range Etest strips and recategorized using MENSURA interpretive criteria. CLSI breakpoints should still be followed when intravenous tobramycin is used in CF patients, particularly during the course of exacerbations.

HIGHLIGHTS:

1. Etest merits further consideration for testing of CF isolates since timely evaluation of the resistance problem within this patient group is needed for adequate therapy since they are usually colonized with multi-resistant strains for which few treatment options exist.
2. The wide and higher concentration range covered by Etest Tobramycin strips give a more precise reflection of the true drug levels reached in pulmonary secretions when delivered by aerosolization.

EXAMPLES OF NOTABLE ETEST® REFERENCES -2006

1. **Evaluation of the Etest for the assessment of synergy of antibiotic combinations against multiresistant *Pseudomonas aeruginosa* isolates from cystic fibrosis patients.** Balke B, Hogardt M, Schmoldt S, Hoy L, Weissbrodt H, Haussler S. *European Journal of Clinical Microbiology & Infectious Diseases*. 2006 Jan;25(1):25-30.

The determination of synergistic effects of antimicrobial drug combinations can lead to improved therapeutic options in the antibiotic treatment of cystic fibrosis patients who are chronically infected with multiresistant *Pseudomonas aeruginosa* isolates. The aim of this study was to evaluate the performance of the Etest versus the standard agar dilution checkerboard susceptibility test in the assessment of synergy and, in addition, to determine the activity of two antimicrobial combinations against 163 multiresistant *P. aeruginosa* isolates from cystic fibrosis patients. The agreement between the checkerboard method and the Etest was excellent (>90%) for nonmucoid as well as mucoid isolates from cystic fibrosis patients. The rate of synergy was higher for the antibiotic combination of ceftazidime and tobramycin (28.8% of the cystic fibrosis strains) than for the combination of meropenem and tobramycin (19.0%). However, the probability of synergy for the second antibiotic combination increased significantly when the synergy of the first antibiotic combination had already been demonstrated (Fischer's exact test, $p=0.049$). The results show that the Etest is a valuable and practical method for routine microbiological diagnostics and can aid in the selection of improved antibiotic options in the treatment of cystic fibrosis patients chronically infected with *P. aeruginosa*.

HIGHLIGHTS:

1. The reproducibility of Etest MIC results was excellent and no serious discrepancies were seen even when MIC values obtained at different laboratories were compared.
2. Comparison of Etest combination testing results with the checkerboard agar dilution method showed an overall agreement >90% for the synergism seen for 163 *P. aeruginosa* CF isolates.
3. Etest was found to be rapid, reproducible, easy-to-perform, and flexible for determining synergistic activity that could significantly contribute to the improvement of therapeutic options in the treatment of chronic *P. aeruginosa* infections.

2. **Amphotericin B and caspofungin resistance in *Candida glabrata* isolates recovered from a critically ill patient.** Krogh-Madsen M, Arendrup MC, Heslet L, Knudsen JD. *Clinical Infectious Diseases*. 2006 Apr 1;42(7):938-44.

Background: Consecutive *Candida glabrata* isolates recovered from a patient in an intensive care unit were resistant to amphotericin B (minimum inhibitory concentration, up to 32 µg/mL; determined by Etest [AB BIODISK]). Analyses at the national reference laboratory showed that some isolates were also resistant to azoles and caspofungin. In this study, 4 isolates were studied thoroughly using susceptibility assays and a mouse model and to determine clonality.

Methods: Different broth microdilution tests, Etests, and time-kill studies for antifungals were performed in different media. Three of the 4 isolates were examined in an *in vivo* experiment, in which mice were challenged intravenously with 1 of 3 isolates and treated daily with amphotericin B, caspofungin, or saline. For the clonality studies, arbitrarily primed polymerase chain reaction (PCR) was performed with the 4 isolates, 8 isolates obtained from non related patients, and a reference strain.

Results: The murine model indicated that 1 isolate was resistant to amphotericin B, 1 had intermediate susceptibility, and 1 was fully susceptible. Two of the 3 isolates were resistant to caspofungin. Microdilution methods did not reliably differentiate between amphotericin B-susceptible and -resistant isolates. All assays identified caspofungin-susceptible and -resistant isolates. Arbitrarily primed PCR showed that the 4 isolates

probably were of clonal origin.

Conclusions: We have documented the emergence of amphotericin B-resistant and caspofungin-resistant *C. glabrata* isolates during treatment of a critically ill liver transplant recipient. Only the Etest predicted amphotericin B resistance in the isolates. We recommend that important fungal strains recovered from patients who are receiving antifungal therapy should be tested for susceptibility to the antifungal drug used, because resistance can be present initially or may occur during treatment.

HIGHLIGHTS:

1. Amphotericin B-resistant and caspofungin-resistant *C. glabrata* isolates can emerge during treatment of critically ill liver transplant recipients.
2. The animal model data proved that some *C. glabrata* isolates were less susceptible and probably had therapeutic resistance to treatment with amphotericin B.
3. Etest was superior to broth microdilution methods in detecting the resistant isolates and it was the only method that could identify the intermediately susceptible isolate.

3. Multicenter evaluation of the Etest and disk diffusion methods for differentiating daptomycin-susceptible from non-daptomycin-susceptible *Staphylococcus aureus* isolates. Jevitt LA, Thorne GM, Traczewski MM, Jones RN, McGowan JE Jr, Tenover FC, Brown SD.

Journal of Clinical Microbiology. 2006 Sep; 44(9):3098-104.

Daptomycin is a novel cyclic lipopeptide that is approved by the U.S. Food and Drug Administration for the treatment of complicated skin and skin structure infections associated with *Staphylococcus aureus* and other gram-positive pathogens and also staphylococcal bacteremia, including right-sided endocarditis. The Clinical and Laboratory Standards Institute (CLSI) established "susceptible-only" interpretive criteria for broth microdilution (BMD) and disk diffusion (DD) testing of daptomycin in 2005. However, a series of *S. aureus* isolates have been recovered with daptomycin MICs in the nonsusceptible range (i.e., MICs of >1 microg/ml). The objective of this study was to determine the ability of the Etest and DD methods to differentiate daptomycin-susceptible from nonsusceptible isolates of *S. aureus* compared to the results of the CLSI BMD reference method. There was a good correlation between Etest MIC results and the results of BMD among laboratories ($r = 0.86$ to 0.88), with 95.3% of the Etest MICs within a $\pm 1 \log(2)$ dilution of the BMD MIC result. A total of 92 of 102 (90.2%) non-daptomycin-susceptible isolates of *S. aureus* identified by BMD in two participating laboratories were also classified as nonsusceptible by Etest. However, the very major and major error rates reported by one of the participating laboratories were 13.5 and 4.0%, respectively, primarily due to the absence of an intermediate category. The DD method, however, did not reliably differentiate daptomycin-susceptible from non-daptomycin-susceptible isolates. In 2005, daptomycin disks were voluntarily removed from the market by Cubist Pharmaceuticals. The disk diffusion breakpoints were subsequently removed from the CLSI M100 standard in 2006.

HIGHLIGHTS:

1. Etest performed well in differentiating daptomycin-susceptible from non-daptomycin-susceptible isolates compared to the broth microdilution reference method.
2. Disk diffusion was not reliable for daptomycin testing and was removed from the market and CLSI method recommendations.

4. Health and economic outcomes of the detection of *Klebsiella pneumoniae*-produced extended-spectrum beta-lactamase (ESBL) in a hospital with high prevalence of this infection. Marra AR, Pereira CA, Castelo A, do Carmo Filho JR, Cal RG, Sader HS, Wey SB.

International Journal of Infectious Disease. 2006 Jan;10(1):56-60.

Introduction: *Klebsiella pneumoniae* is of high prevalence in hospital infections, mainly in bloodstream infections (BSI), and some produce extended-spectrum beta-lactamase (ESBL). For hospitals with a high prevalence of strains producing this enzyme, there is no reference material to show whether the use of the Etest method for their detection, which can be quite expensive, is actually required.

Objective: To evaluate the cost-benefit of the disk diffusion and Etest methods for the detection of ESBL-producing *K. pneumoniae* strains in hospitals where a high prevalence of this resistance mechanism in BSI is found.

Methods: One hundred and eight patients with *K. pneumoniae* BSI were evaluated retrospectively. ESBL-producing strains were identified by the disk diffusion method and by the Etest method. We estimated the costs of both diagnostic methods based on antimicrobial therapy adequacy.

Results: Fifty-two percent of *K. pneumoniae* infections were due to ESBL-producing strains. The disk diffusion method yielded a positive predictive value (PPV) of 94.7% (95% CI: 88.9-100%) and a negative predictive value (NPV) of 96.1% (CI 95%: 90.8-101.4%) in relation to the Etest. We evaluated cost-effectiveness, i.e., we analyzed the cost of both Etest and disk diffusion methods with carbapenem and cephalosporins, and found that the use of the disk diffusion method accounts for approximately US\$3300.

Conclusions: In hospitals with a high prevalence of ESBL-producing strains, the disk diffusion method can be used to detect ESBL-producing *K. pneumoniae* without compromising the clinical progression of patients with BSI. The Etest showed higher accuracy but this method was more expensive than the disk diffusion method. However, the use of the Etest method was demonstrated to be more cost-effective, as we evaluated cost based on antimicrobial therapy adequacy.

HIGHLIGHTS:

1. In sites of low prevalence of ESBL-producing *K. pneumoniae*, the disk diffusion test shows low positive predictive value.
2. The cost of adequate treatment with carbapenems extends beyond the cost of the diagnostic methods for the detection of ESBL-producing strains.
3. Etest showed higher accuracy but this method was more expensive than the disk diffusion method.
4. Etest was more cost-effective when evaluated based on antimicrobial therapy adequacy

EXAMPLES OF NOTABLE ETEST[®] REFERENCES -2007

1. **Direct Etest (AB BIODISK) of respiratory samples improves antimicrobial use in ventilator-associated pneumonia.** Bouza E*, Torres MV, Radice C, Cercenado E, de Diego R, Sanchez-Carrillo C, and P Munoz.

Clinical Infectious Diseases, February 2007, Vol. 44, No. 3 p.382-387.

Background: Ventilator-associated pneumonia is the most frequently observed nosocomial infection in intensive care units, and it is associated with high morbidity and mortality. Early microbiological diagnosis and the initial administration of appropriate antimicrobial therapy are associated with decreased mortality and potentially reduced costs. Our study evaluates the clinical and financial impact of performing rapid antimicrobial susceptibility tests directly on samples obtained from the lower respiratory tract.

Methods: A prospective, randomized study was performed over a 2-year period. Patients who had a lower respiratory tract infection that was acquired during mechanical ventilation and for whom samples obtained from the respiratory tract were sent for culture were randomized to 1 of 2 groups. Samples were cultured for the control group, and results were reported using standard procedures. Samples were also cultured for the test subject group using standard procedures, but in addition, a rapid antibiogram was immediately performed by placing Etest antibiotic strips (AB BIODISK) directly on respiratory tract samples. Patients in the Etest group received a preliminary laboratory report when it became available. The 2 patient groups were compared according to the following variables: type and severity of underlying conditions, total days of antimicrobial use, number of defined daily doses, cost of acquisition of the antimicrobial agent per episode, days of fever, days receiving mechanical ventilation, days in the intensive care unit, incidence of

Clostridium difficile-associated diarrhea, and mortality.

Results: Reporting a rapid Etest was associated with fewer days of fever, fewer days of antibiotic administration until resolution of the episode of ventilator-associated pneumonia, decreased antibiotic consumption, less *C. difficile*-associated diarrhea, lower costs of antimicrobial agents, and fewer days receiving mechanical ventilation.

Conclusions: A rapid Etest of respiratory tract samples improves antimicrobial use in cases of ventilator-associated pneumonia.

HIGHLIGHTS:

1. An early report based on 6 Etest strips used for direct testing of LRT secretion samples is associated with better antibiotic use, less misuse and a decrease in antimicrobial-related adverse events.
2. The Etest method does not depend on inoculum size and makes it ideal for direct testing of clinical secretion samples where the inoculum can vary.
3. Determining a direct MIC antibiogram with the clinical sample without waiting for bacterial isolation, may provide preliminary information that has very good correlation with standard procedures.
4. VAP patients who were managed using the rapid direct Etest method were associated with:
 - fewer days of fever per episode
 - fewer days of antibiotic administration to resolve the VAP episode
 - decreased antibiotic consumption
 - less *C. difficile* –associated diarrhea
 - lower costs of antimicrobial agents
 - fewer days receiving mechanical ventilation after receiving diagnosis of VAP
 - considerable decrease in antimicrobial misuse
 - important cost savings
6. Performance of the rapid Etest direct VAP specimen testing is very simple and is within the reach of any microbiology laboratory.

2. Evaluation of direct Etest on lower respiratory tract samples: a rapid and accurate procedure for antimicrobial susceptibility testing. Cercenado E, Cercenado S, Marín M, Rico MV, Vicente T, and E Bouza. *Diagnostic Microbiology and Infectious Diseases*. 2007, In press.

We compared the direct Etest susceptibility testing (DET) on respiratory samples with a standard microbroth dilution method (MBD) after quantitative cultures. A total of 152 samples from intensive care unit patients were processed by DET onto Mueller-Hinton agar. Oxacillin, piperacillin/tazobactam, cefepime, imipenem, ciprofloxacin, and amikacin were the antimicrobials evaluated. Cultures were 102 monomicrobials and 50 polymicrobials. Overall, 93.8% of the isolates were recovered by the DET. Among the 772 microorganism-antibiotic combinations evaluated, there was a total agreement with the MBD in 96.1%. There were 8 very major errors (1.03%), 15 major (1.94%), and 7 minor (0.91%). All discrepancies but one corresponded to polymicrobial cultures, and most occurred with cefepime (8 cases, 7.07%) and imipenem (7 cases, 6.18%). Readings of DET were easy to interpret and improved with transmitted light. DET on respiratory samples is a rapid technique that provides susceptibility results in 18 to 24 h comparable with those obtained by MBD.

HIGHLIGHTS:

- 1.
- 2.
- 3.

3. **Prevalence and characteristics of heteroresistant vancomycin-intermediate *Staphylococcus aureus* bacteremia in a tertiary care center.** Maor Y, Rahav G, Belasov , Ben-David D, Smollan G, Keller N. *Journal of Clinical Microbiology*.2007 May;45(5):1511-4.

Infections with *S. aureus* with heterogeneous intermediate resistance to vancomycin (hVISA) are occurring more frequently. The detection of these infections, their prevalence, clinical characteristics, and significance are controversial. During 2003 and 2004, all blood culture isolates of methicillin-resistant *Staphylococcus aureus* (264 patients) at the Sheba Medical Center, Tel Hashomer, Israel, were assessed for hVISA by using the Etest macromethod. A total of 16 patients (6%) were positive for hVISA. Resistance to teicoplanin alone and to vancomycin alone using the Etest macromethod was found in 14 and 10 patients, respectively. Standard MICs to vancomycin were between 1 to 4 mg/ml. Most of these isolates (12 of 16 [75%]) would have been missed without specific testing. The median number of bacteremic days was 4. Seven patients had positive blood cultures for more than 5 days. Twelve patients died, and for eight of these the deaths were directly related to hVISA sepsis. We found that hVISA bacteremia was prevalent in our institution, and we suggest seeking hVISA in patients with persistent *S. aureus* bacteremia.

HIGHLIGHTS:

1. “we have demonstrated that hVISA isolates are more common than previously thought.
2. Detecting these isolates necessitates specific laboratory diagnosis even when the new CLSI guidelines for detecting VISA are used.
3. We propose to incorporate the Etest macromethod routinely since this information is clinically important.”

4. **Acquired resistance to echinocandins in *Candida albicans*: case report and review.** Baixench MT, Aoun N, Desnos-Ollivier M, Garcia-Hermoso D, Bretagne S, Ramires S, Piketty C, Dannaoui E. *Journal of Antimicrobial Chemotherapy*. 2007 Jun;59(6):1076-83.

Objectives: A patient with *Candida albicans* thrush and oesophagitis was treated with high doses of caspofungin but treatment eventually failed. Four *C. albicans* isolates were serially recovered before and after caspofungin treatment. A microbiological study was performed to characterize these four isolates. Methods In vitro antifungal susceptibility testing was performed by the EUCAST reference method in RPMI and AM3 and by Etest((R)). Molecular typing of the four isolates was done by sizing three polymorphic microsatellite markers. To look for specific mutations, sequencing of a region of the gene encoding the 1-3-beta-d-glucan synthase was performed for the four isolates.

Results: In vitro antifungal susceptibility testing showed an increase in both caspofungin and micafungin MICs for the two isolates recovered after caspofungin treatment failure. The best discrimination between the pre-treatment and post-treatment isolates was obtained with Etest((R)). Molecular typing of the four isolates showed that the post-treatment isolates with reduced susceptibility were identical to a susceptible pre-treatment isolate, suggesting the acquisition of caspofungin resistance. Sequencing of the gene encoding the 1-3-beta-d-glucan synthase showed a mutation responsible for an amino acid change at Phe-641 that could confer reduced susceptibility to both echinocandins.

Conclusions: Our results indicate that it is useful to perform in vitro susceptibility testing in the cases of clinical failure during caspofungin therapy.

HIGHLIGHTS:

1. “In the present case, acquisition of resistance was demonstrated in a patient treated with caspofungin. Clinical resistance was associated with increased MICs in vitro and related to a new mutation of the target enzyme.”
2. “the discrimination between pre- and post-treatment isolates was much better with Etest®, suggesting that Etest® could be a reliable and efficient technique to detect *Candida* spp. isolates with reduced susceptibility to caspofungin.”

3. “acquisition of caspofungin resistance in the present isolates was associated with an increase in micafungin MIC, suggesting cross-resistance between these two echinocandins.”
4. “The Etest® method allowed easy detection of elevated MIC, suggesting that it could be used to perform susceptibility testing in cases of clinical failure during caspofungin therapy.”

4. Comparison of Etest with agar dilution for testing the susceptibility of *Pseudomonas aeruginosa* and other multidrug-resistant bacteria to colistin. Goldstein FW, Ly A, Kitzis MD.

Journal of Antimicrobial Chemotherapy. 2007 May;59(5):1039-40.

Colistin, introduced for clinical use > 45 years ago, has not been widely prescribed because of an alleged poor activity and high toxicity.¹ The increasing prevalence of multidrug-resistant Gram-negative bacteria has stimulated the potential indication of colistin in life-threatening infections. In recent studies, colistin has been considered as an effective and less toxic compound than in older studies, under the conditions of modern patient care and drug monitoring.^{1,2} Colistin is used parenterally as sodium colistin methanesulfonate, which is less toxic than the locally used colistin sulphate, despite having less favourable pharmacokinetic properties.¹ However, colistin-resistant mutants can be easily selected *in vitro*,³ particularly in *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, with a frequency of 10⁻⁶ cfu/mL. In the latter species, this could be related to the presence of heteroresistance.¹

As already published 38 years ago,⁴ and occasionally ‘rediscovered’, colistin diffuses poorly in agar, resulting in highly unreliable disc tests; up to 89% of results were categorized as very major discrepancies in a recent study, emphasizing the need for alternative testing methods.⁵

The Etest is a simple and accurate method for determining antibiotic susceptibility. However, it has not been validated against a large number of strains with acquired colistin resistance: in a recent study with *A. baumannii*, poor results have been observed for resistant strains.⁶

The aim of our study was to evaluate colistin Etests versus agar dilution against colistin-susceptible and -resistant clinical isolates

HIGHLIGHTS:

1. “antibiotic susceptibility testing is still performed in many laboratories by the disc diffusion technique, which is highly unreliable for colistin.
2. We strongly recommend that colistin MICs should be determined by Etest for all patients with severe infections caused by multidrug-resistant strains or in cases of clinical failure, and we emphasize the need to transmit these results to the clinician.”