



感受性測試的原則與方法： 紙錠法、最低抑菌濃度法

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課程大綱

1.Principle of AST Performance

2.Method of AST

3.Reading and Interpretation of AST

4.Reporting of AST

5.結論



1.Principle of AST Performance



抗微生物製劑敏感性試驗(AST)

- 可預測或監測治療的有效性
 - 正確的檢測臨床相關的個別菌株之抗藥性
 - 預測抗藥性失敗時將導致病人罹病率或死亡率的增加

因此僅需針對造成感染的分離菌株進行試驗



實驗室需要了解AST哪些事情？

- 哪些菌株要去測試？
- 用什麼方法來做？
- 要測試哪些抗生素？
- 如何報告結果？



血液培養檢體

- 報告病原菌的鑑定和藥敏結果
- 若相同的菌種有兩種不同的形態，應相對報告兩種藥敏結果。
- 疑似污染的菌株不要操作藥敏試驗
 - 但疑似污染的菌株要被保存，尤其是在同一病人分離出來自不同套的檢體時，應考慮進行藥敏試驗。



資料來源：Baron. Cumitech 1C, Blood Cultures. 2005.
CLSI. M47-A; Principles and Procedures for Blood Cultures. 2007.

痰檢體(1/3)

菌量	菌屬(種)	
呼吸道常在菌	Viridans group streptococci, commensal <i>Neisseria</i> spp., diphtheroids, CoNS, <i>Rothia</i> , group F streptococci, anaerobes, <i>Haemophilus</i> spp. (not <i>H. influenzae</i>), enterococci, <i>Candida</i> spp., <i>Eikenella</i> , <i>Actinobacillus</i> , <i>Capnocytophaga</i> , and <i>Moxaxella</i> .	
絕對致病菌 (任何菌量皆須操作)	<ol style="list-style-type: none"> 1. <i>S. pyogenes</i> 2. <i>Brucella</i> spp. 3. <i>Francisella tularensis</i> 4. <i>Yersinia pestis</i> 5. <i>Bacillus anthracis</i> 	<ol style="list-style-type: none"> 6. <i>Rhodococcus equi</i> 7. <i>Streptomyces</i> 8. <i>Nocardia</i> 9. <i>Cryptococcus neoformans</i> 10. non-saprophytic molds



資料來源：台灣醫事檢驗學會：痰液檢體診斷下呼吸道感染—草案版 (2011)

痰檢體(2/3)

菌量	菌屬(種)	
<p>可能致病菌 (中等量至大量的菌量且大於呼吸道常在菌量)</p>	<ol style="list-style-type: none"> 1. <i>S. pneumoniae</i> 2. <i>S. aureus</i> 3. <i>M. catarrhalis</i> 4. <i>H. influenzae</i> 5. <i>N. meningitides</i> 6. Group B, C, G streptococci 7. <i>Pasteurella</i> spp. 8. 1 or 2 types Enterobacteriaceae 	<ol style="list-style-type: none"> 9. <i>Bordetella bronchiseptica</i> 10. <i>S. maltophilia</i> 11. <i>Acinetobacter</i> 12. <i>P. aeruginosa</i> 13. <i>B. cepacia</i> 14. Other aerobic gram-negative bacilli 15. Urea (+) <i>Corynebacterium</i>
<p>可能致病菌大於二種以上 (中等量至大量的菌量且大於呼吸道常在菌量)</p>	<ol style="list-style-type: none"> 1. 觀察抹片，若完全無上皮細胞，至多操作三種菌。 2. 若有1-9/LPF的上皮細胞且白血球大於25/LPF，操作抹片中與PMN有關係至多二種菌。 	



資料來源：台灣醫事檢驗學會：痰液檢體診斷下呼吸道感染—草案版 (2011)

痰檢體(3/3)

- 痰液在培養基上有意義的菌量為：
 - 生長菌量為中等量以上或長於第二區以上。
 - 少量的致病菌但於革蘭氏染色觀察中是一致且與PMN有關係。
 - 菌落僅生長在第一區或沒有呼吸道常在菌的純致病菌菌落(Pure growth of a primary pathogen)並且革蘭氏染色中與PMN有關係。



資料來源：台灣醫事檢驗學會：痰液檢體診斷下呼吸道感染—草案版 (2011)

糞便檢體

- 腸道致病菌進行藥敏試驗
- 鑑定數量較多的 *P. aeruginosa* 或 *S. aureus*，但不操作藥敏試驗。
- 報告生長量多的酵母菌，但不操作藥敏試驗。



資料來源：Lynne S. Garcia. 2010. Clinical Microbiology Procedure Handbook, 3rd ed. ASM press.

尿液檢體(1/2)

- 尿液之微生物菌叢

微生物菌叢(Microbiota)	菌種(Organism)
泌尿生殖道常在菌(Urogenital microbiota)	Viridans group streptococci, <i>Neisseria</i> spp., diphtheroids, <i>Lactobacillus</i> spp. anaerobes
皮膚常在菌(Skin microbiota)	Diphtheroids, <i>Staphylococcus</i> spp.
泌尿道致病菌(Uropathogens)	Beta-hemolytic streptococci, <i>Enterococcus</i>



資料來源：台灣醫事檢驗學會：痰液檢體診斷下呼吸道感染—草案版 (2011)

尿液檢體(2/2)

採檢方式	定量	1 株菌	2 株菌(泌尿道致病菌)	≥ 3 株菌(泌尿道致病菌)
中段尿	0.001- mL	當<10,000 CFU/mL時，報告染色與菌落定量結果； 當≥10,000 CFU/mL時，鑑定並做藥物感受性試驗。 當檢體來自女性時，≥1,000 CFU/mL的泌尿道致病菌(<i>GBS</i> 和 <i>S. saprophyticus</i>)需要鑑定。	任一株<100,000 CFU/mL時，報告染色與菌落定量結果； 任一株≥100,000 CFU/mL時，鑑定並做藥物感受性試驗。	有主要量多的(≥100,000 CFU/mL)泌尿道致病菌時，鑑定並做藥物感受性試驗，最多做2種。 若3種(含)以上的泌尿道致病菌，定量均≥100,000 CFU/mL者或無主要量多的泌尿道致病菌時，報告染色和定量結果，並註明「疑似污染，臨床上有需要的話，請再重新採檢開單送驗。」
導尿		當<10,000 CFU/mL時，報告染色與菌落定量結果； 當≥10,000 CFU/mL時，鑑定並做藥物感受性試驗。		
單次導尿 小孩導尿 恥骨上穿刺 腎臟尿 腎臟造瘻管尿 膀胱鏡尿	0.01- mL	當≥1,000 CFU/mL或任何純培養的泌尿道致病菌時，鑑定並做藥物感受性試驗。 當泌尿生殖道或皮膚常在菌存在時，100-1,000 CFU/mL的泌尿道致病菌需報告染色與菌落定量結果。	當<1,000 CFU/mL時，報告染色與菌落定量結果； 當≥1,000 CFU/mL的致病菌時，鑑定並做藥物感受性試驗。	任一株<10,000 CFU/mL時，報告染色與菌落定量結果； 任一株≥10,000 CFU/mL時，鑑定並做藥物感受性試驗。或與醫師聯繫需做哪些菌株。



資料來源：台灣醫事檢驗學會：痰液檢體診斷下呼吸道感染—草案版 (2011)

傷口檢體

- 如果革蘭氏染色中發現細菌存在於白血球的關係，操作鑑定與藥敏試驗至多3株。
- 對於皮膚的微生物（類白喉桿菌，凝固酶陰性葡萄球菌，草綠色鏈球菌），僅於下列狀況執行ID和AST。
 - 從侵入性的部位收集的檢體
 - 從革蘭氏染色中確定為良好的開放性傷口檢體



資料來源：Lynne S. Garcia. 2010. Clinical Microbiology Procedure Handbook, 3rd ed. ASM press.
Bowler PG,. 2001. Clin Microbiol Rev.:(14): p. 244-269.



2.Method of AST



AST Method

- Disk Diffusion Method
- Dilution Method
 - Agar Dilution
 - Broth (micro- or macro-) method
 - Antimicrobial gradient method
 - Breakpoint susceptibility testing
 - Instrument-based methods
- Resistance screens
- Beta-Lactamase tests
- Detection of enzymes or genes for resistance

Disk Diffusion Method (Kirby-Bauer Method)



Drs. Bauer and Kirby



Inoculation Procedures



Select colonies



Prepare inoculum suspension



Standardize inoculum suspension to McFarland



Inoculum Preparation(1/2)

- Select inoculum preparation
 - Direct colonies suspension
 - Growth slowly or unpredictably in broth
 - Staphylococci
 - Fresh (18-24 hrs) growth is available
 - log phase (35 °C , 2-8 hours)
 - Nonfastidious rapidly growing bacteria when fresh colonies are not available



Inoculum Preparation(2/2)

- 3-5 well isolated colonies with broth (TSB) or 0.9% NaCl
- Inoculum density : McFarland No. 0.5 (1.5×10^8 CFU/mL)



1. Inoculation of agar plate
2. Application of disks
3. Incubation



Invert and incubate plates

Incubating The Plate

- 非挑剔性的細菌培養於at 35 °C 16-18小時
– 24 Hours-*Enterococcus* vs. Vancomycin,
Staphylococcus vs. Oxacillin and Vancomycin
- 挑剔性細菌推薦培養的條件如下

Bacteria	Medium	Time (h)	atmosphere
<i>Haemophilus spp.</i>	HTM	16-18	CO ₂ (5%)
<i>N. gonorrhoeae</i>	GC agar+1% sup.	20-24	CO ₂ (5%)
<i>S. pneumoniae</i>	MHA-5% S.B	20-24	CO ₂ (5%)
Other <i>Streptococcus</i> spp.	MHA-5% S.B	20-24	CO ₂ (5%)

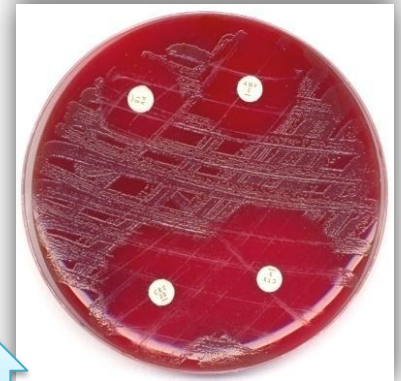
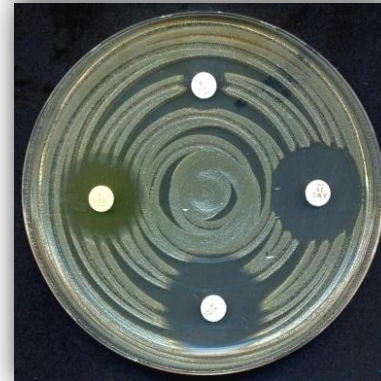
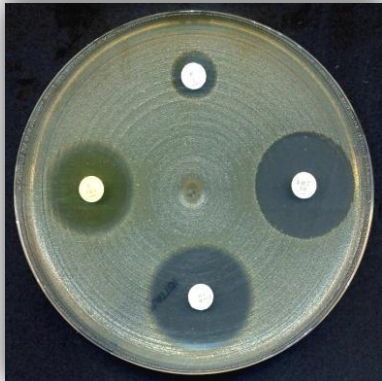
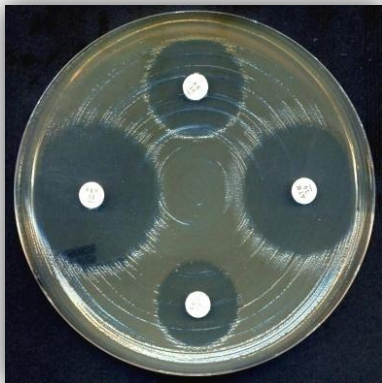
Summary of Inoculation Process

- 15-15-15 minute rule
 - 從隔夜非選擇性培養基上的單一菌落作成懸浮液。
 - 調整濃度於McFarland 0.5，最好使用測光裝置。理想情況下，在15分鐘內使用接種。
 - 使用無菌棉棒浸入到溶液中，在管內旋轉棉棒去除多餘的液體。
 - 接種於整過培養基表面。
 - 15分鐘內貼上紙錠並於15分鐘內放入培養箱。



資料來源：EUCAST 2013 Version 3.0

均勻接種的培養基生長情況



Plates should look like this..

..and NOT like this!



資料來源：EUCAST 2013 Version 3.0



Affect of Results

- 培養基成分
- 培養基pH值
- 濕度
- 瓊脂深度
- 接種的濃度
- 接種的過程
- 抗生素紙錠的濃度
- 紙錠的儲存
- 紙錠貼在培養皿上的數量
- 培養溫度
- 培養條件

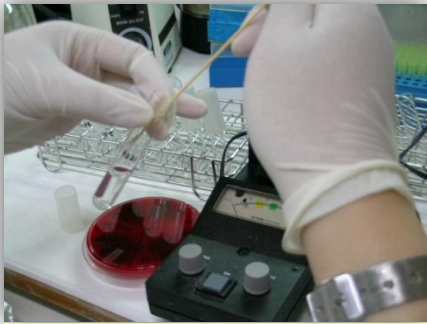


Dilution Methods

- Agar dilution
- Broth dilution
 - Micro-dilution
 - Macro-dilution
- Antimicrobial gradient method
- Breakpoint susceptibility testing
- Instrument-based methods

Minimum Inhibitory Concentrations

Agar Dilution



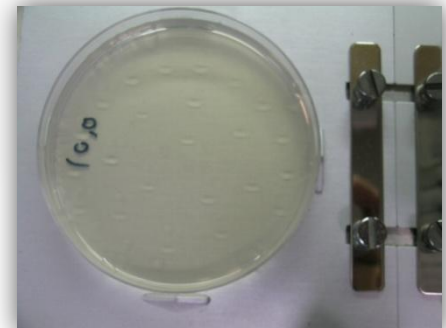
Prepare inoculum suspension



10^7 CFU/mL of the suspension to wells

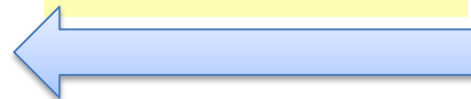


1-2 μ l of the suspension of 10^7 CFU/mL is delivered to agar surface



Final desired inoculum of approximately 10^4 CFU per spot

Incubation and Reading



Broth Micro-dilution



Two fold of 100 μ l diluted agents into wells



0.002 mL suspension
+ 2 mL MHB

10^5 CFU/mL



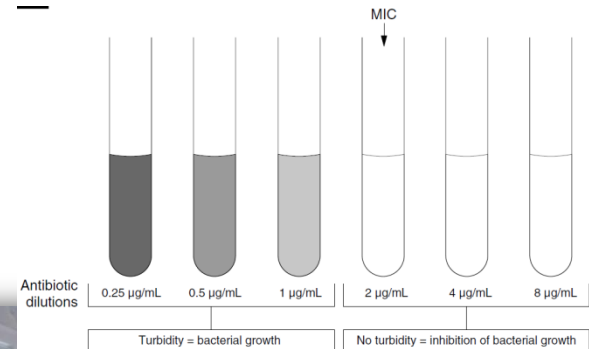
10 μ l 10^5 CFU
suspension + 100
 μ l agents



10^5 CFU/mL

Mixing

Incubation
and
Reading



Antimicrobial Gradient Method

E-test

- 培養基和接種法如同紙錠擴散法
- Application of E-test strips
 - Only one or two strips for 100 mm plates
 - 1-6 strips for 150 mm plates
 - Radiating from the center, the “E” end point to rim of the petri dish
 - Remove air large bubble underneath
 - Do not relocate an E strip once landed on the agar surface

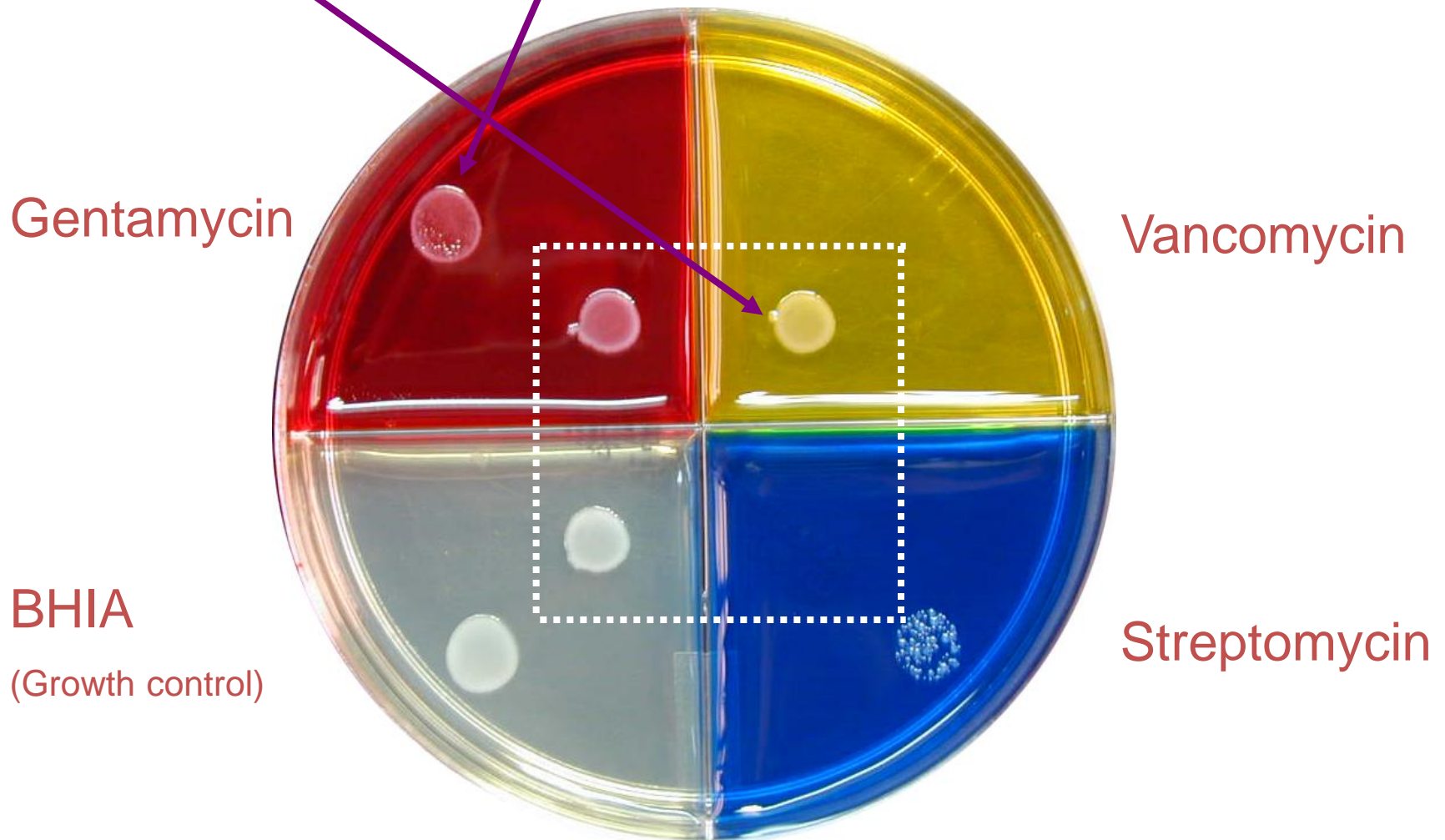




HLAR and VA Screen Testing

- Inoculum preparation
 - Transfer colonies to broth or saline and adjust approximately 0.5 McFarland.
- Inoculation and incubation
 - Using a 1 μ l or 10 μ l (HLAR for 10 μ l) loop dipped in suspension, spot onto plate surface to cover 10-15 mm in diameter.
 - Invert plates and incubate at 35°C in ambient-air
 - Examine after overnight, If susceptible full 24 hrs incubation.

VRE & HLAR Screen Agar



MRSA Screening Testing(1/2)

- Inoculum preparation
 - Transfer colonies to broth or saline and adjust approximately 0.5 McFarland

- Inoculation and incubation

- Method 1

- Using a 1ul loop dipped in suspension, spot onto plate surface to cover 10-15 mm in diameter

- Method 2

- Dip a sterile cotton swab into suspension and express fluid the side of the tube
- Spot onto plate surface to cover 10-15 mm in diameter



MRSA Screening Testing(2/2)

- Inoculum preparation
 - Transfer colonies to broth or saline and adjust approximately 0.5 McFarland
- Inoculation and incubation
 - Invert plates and incubate at 35°C in ambient-air
 - Examine after overnight, If susceptible full 24 hrs incubation



Beta-lactamase Testing

- Chromogenic cephalosporin method
 - 放置Cefinase紙錠在玻片或培養皿蓋上
 - 用1滴無菌蒸餾水濕潤紙錠
 - 使用無菌接種環挑取菌落塗抹在紙錠上
 - 在15秒到5分鐘內觀察顏色的改變





Automated Instrument Systems

- Standardize reading of end points
- Produce results shorter period than manual readings, generate rapid (3.5–16 h)
- Affect of Results
 - Colonies growth time (log phase)
 - Pure colonies
 - Concentration of inoculum
 - Kit storage

Instrument Method(1/2)

VITEK 2

- 操作時應注意事項：

- 調製菌液請使用VITEK 2 DensiChek（濁度儀）來確定菌液濃度。
- 如果卡片的鋁箔外包裝破損，請勿使用。
- 調製菌液之菌落取自BAP、CNA、MAC (GN)等培養基。
- 使用乾淨的透明塑膠材質試管(12x75mm)，避免使用玻璃材質的試管。
- 不要穿戴有粉末的手套來調製菌液。
- 卡片保存在2- 8°C。
- 以0.45-0.5%，pH5.0-7.2的無菌食鹽水來調製菌液。

bioMérieux

Instrument Method(2/2)



Pheonix 100

- 操作時應注意事項：
 - 打開包裝後，測試盤(Panel)需在2小時內使用，勿使用過期之卡片。
 - 菌落取自BAP、Chocolate、CNA、MAC和EMB (GN)等培養基。
 - 調整菌液濃度，不可使用Polyester材質棉棒，可能影響判讀結果。
 - AST Indicator需回溫到室溫才可使用，依規定時間內使用與儲存。
 - 接種完成30分鐘內（必須垂直放置），需將試劑組裝載入機器。
 - 接種完成的試劑組需小心處理，避免碰撞或震動。

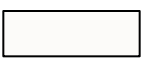
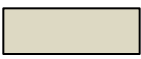


Becton Dickinson



3. Reading and Interpretation of AST



Suggested Antimicrobial Agents for Routine Testing and Reporting From CLSI (1/7)

- 『 * 』 MIC testing only ; disk diffusion test unreliable
- 『 † 』 Routine testing is not necessary
- “ or ” can be to predict results for other agent
-  Group A primary test and report
-  Group B primary test report selectively
-  Group C supplemental report selectively
-  Group U supplemental for urine only



資料來源：CLSI, M100-S23



Suggested Antimicrobial Agents for Routine Testing and Reporting From CLSI (2/7)

<i>Enterobacteriaceae</i>		<i>P. aeruginosa</i>
Ampicillin	Piperacillin	Ceftazidime
Cefazolin	SXT	Gentamicin, tobramycin
Gentamicin, tobramycin	Aztreonam, ceftazidime	Piperacillin
Amikacin	Ceftaroline	Amikacin
AMC, SAM, TZP, TIM	Chloramphenicol	Aztreonam
Cefuroxime	Tetracycline	Cefepime
Cefepime	Cephalothin	Ciprofloxacin, levofloxacin
Cefotetan, cefoxitin	Lomefloxacin or ofloxacin Norfloxacin	Doripenem, imipenem, meropenem
Cefotaxime or ceftriaxone	Nitrofurantoin	TZP, Ticarcillin
Ciprofloxacin, levofloxacin	Sulfisoxazole	Lomefloxacin or ofloxacin Norfloxacin
Doripenem, ertapenem Imipenem, meropenem	Trimethoprim	



資料來源：CLSI, M100-S23



Suggested Antimicrobial Agents for Routine Testing and Reporting From CLSI (3/7)

<i>Staphylococcus spp.</i>		<i>Enterococcus spp.</i>
Azithromycin or clarithromycin or erythromycin	Doxycycline, Minocycline, Tetracyclinea	Ampicillin, Penicillin
Clindamycin	Rifampin	*Daptomycin
Oxacillin (cefoxitin)	Chloramphenicol	Piperacillin
Amikacin	Ciprofloxacin or LVX or OFX Moxifloxacin	Linezolid
Penicillin	Gentamicin	Vancomycin
SXT		Gentamicin (high-level)
Ceftaroline	Lomefloxacin, Norfloxacin	Streptomycin (high-level)
*Daptomycin	Nitrofurantoin	CIP, LVX, Norfloxacin
Linezolid	Sulfisoxazole	Nitrofurantoin
*Vancomycin	Trimethoprim	Tetracycline



資料來源：CLSI, M100-S23



Suggested Antimicrobial Agents for Routine Testing and Reporting From CLSI (4/7)

<i>Acinetobacter</i> spp.	<i>Burkholderia cepacia</i>	<i>S. maltophilia</i>
SAM	SXT	SXT
Ceftazidime	Ceftazidime	*Ceftazidime
Ciprofloxacin, Levofloxacin	*Chloramphenicol	*Chloramphenicol
Imipenem, Meropenem	*Levofloxacin	Levofloxacin
Gentamicin, Tobramycin	Meropenem	Minocycline
Amikacin	Minocycline	*TIM
TZP, TIM	*TIM	
Cefepime		
Cefotaxime, Ceftriaxone		
Doxycycline, MI, Tetracycline		
Piperacillin		
SXT		



資料來源：CLSI, M100-S23



Suggested Antimicrobial Agents for Routine Testing and Reporting From CLSI (5/7)

<i>Haemophilus influenzae</i> and <i>H. parainfluenzae</i>		<i>Neisseria gonorrhoeae</i>
Ampicillin	Cefdinir or cefixime or cefpodoxime	[†] Ceftriaxone, [†] Cefixime
SXT	Ceftaroline	[†] Ciprofloxacin
SAM	Cefuroxime (oral)	[†] Tetracycline
Cefuroxime (parenteral)	Ertapenem or imipenem	
Cefotaxime or ceftazidime or ceftriaxone	Ciprofloxacin or levofloxacin or lomefloxacin or moxifloxacin or ofloxacin, Gemifloxacin	
Chloramphenicol	Rifampin	
Meropenem	Telithromycin	
Azithromycin, Clarithromycin	Tetracycline	
AMC		



資料來源：CLSI, M100-S23

Suggested Antimicrobial Agents for Routine Testing and Reporting From CLSI (6/7)

<i>Streptococcus pneumoniae</i>		*Other Non- <i>Enterobacteriaceae</i>
Erythromycin	*Amoxicillin, *AMC	Ceftazidime
Penicillin (oxacillin disk)	*Cefuroxime	Gentamicin, Tobramycin
SXT	Ceftaroline	Piperacillin
*Cefepime, *Cefotaxime, *Ceftriaxone	Chloramphenicol	Amikacin, Aztreonam, Cefepime TZP, TIM, SXT,
Clindamycin	Linezolid	Ciprofloxacin, Levofloxacin
Gemifloxacin, Levofloxacin Moxifloxacin, Ofloxacin	*Ertapenem, *Imipenem	Imipenem, Meropenem Cefotaxime, Ceftriaxone
*Meropenem	Rifampin	Chloramphenicol
Telithromycin		Lomefloxacin or ofloxacin Norfloxacin
Tetracycline		Sulfisoxazole
Vancomycin		Tetracycline



資料來源：CLSI, M100-S23



Suggested Antimicrobial Agents for Routine Testing and Reporting From CLSI (7/7)

<i>Streptococcus</i> spp.β-Hemolytic	<i>Streptococcus</i> spp. Viridans Group
Clindamycin	*Ampicillin, *Penicillin
Erythromycin	Gentamicin, Tobramycin
†Penicillin or †ampicillin	Piperacillin
Cefepime or Cefotaxime or Ceftriaxone	Cefepime , Cefotaxime , Ceftriaxone
Vancomycin	Vancomycin
Ceftaroline	Clindamycin
Chloramphenicol	Chloramphenicol
*Daptomycin	Erythromycin
Levofloxacin, Ofloxacin	
Linezolid	Linezolid



資料來源：CLSI, M100-S23



Don't Report in AST

Organism	Antimicrobial Agents That Must Not Be Reported as Susceptible
<i>Salmonella</i> spp., <i>Shigella</i> spp.	1st- and 2nd-generation cephalosporins, cephamycins, and aminoglycosides
MRS	Penicillins, β -lactam/ β -lactamase inhibitor combinations, antistaphylococcal cephems (except cephalosporins with anti-MRSA activity), and carbapenems
<i>Enterococcus</i> spp.	Aminoglycosides (except high conc.), cephalosporins, clindamycin, and trimethoprim-sulfamethoxazole

Daptomycin should not be reported for isolates from the respiratory tract.



資料來源：CLSI, M100-S23



AST for Infrequently Isolated or Fastidious Bacteria

- *Abiotrophia* spp. and *Granulicatella* spp.
- *Aeromonas* spp and *Plesiomonas shigelloides*
- *Bacillus* spp. (Not *B. anthracis*)
- *Campylobacter jejuni/coli*
- *Corynebacterium* spp.
- *Erysipelothrix rhusiopathiae*
- HACEK Group
- *Helicobacter pylori*
- *Lactobacillus* spp.
- *Leuconostoc* spp.
- *Listeria monocytogenes*
- *Moraxella catarrhalis*
- *Pasteurella* spp.
- *Pediococcus* spp.
- *Vibrio* spp.

CLSI M45

M45-A2
Vol. 30 No. 18
Replaces M45-A
Vol. 26 No. 19

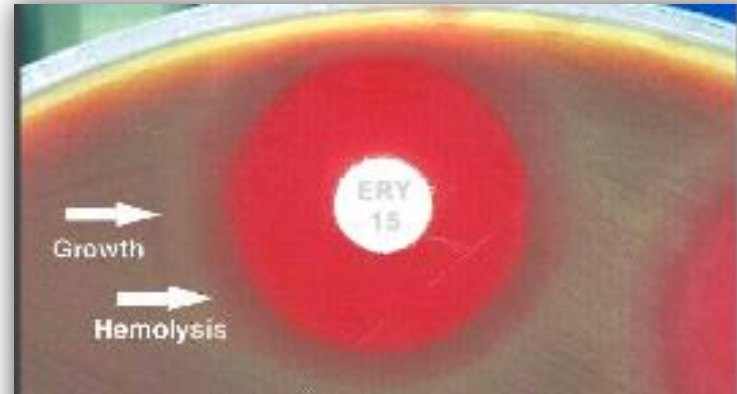
Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria; Approved Guideline—Second Edition



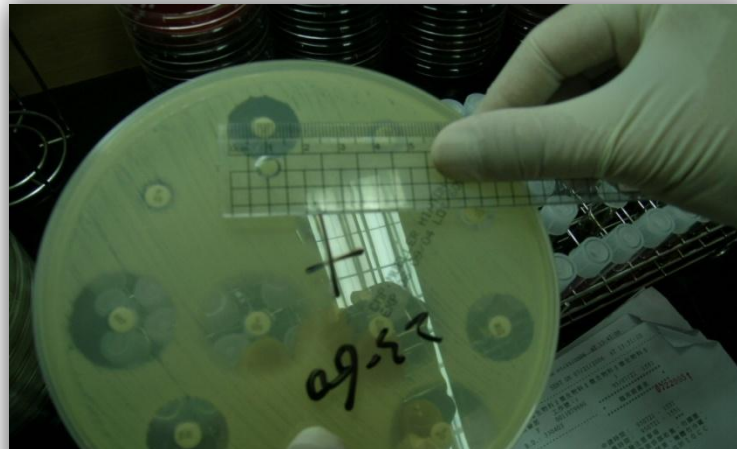
Reading and Interpretation

Susceptible (S), Intermediate (I), Resistant (R)

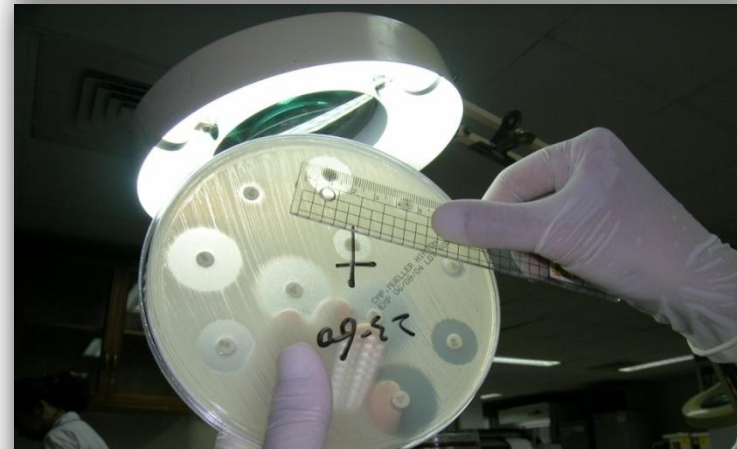
Reading Zone Size



Zone of growth, not hemolysis



Reflected light



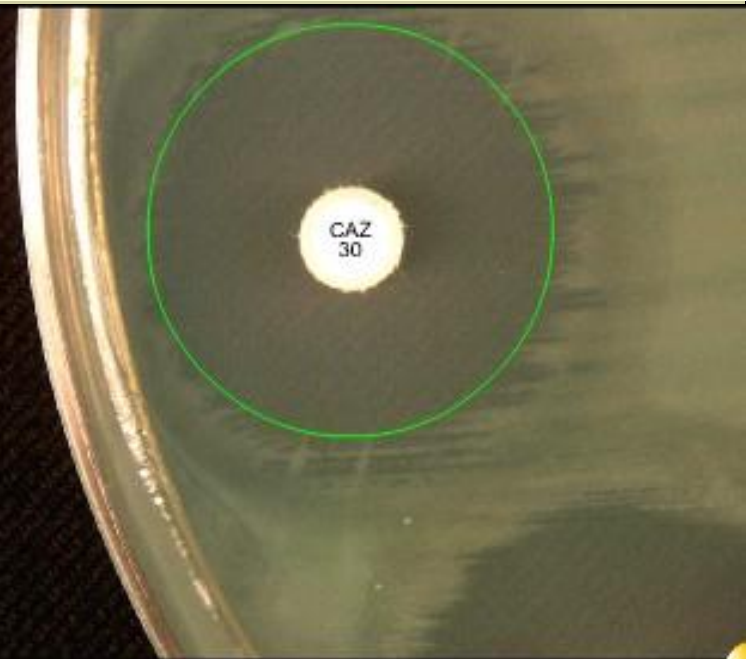
Transmitted light (For VA, OX)

Zone with Feathered Edge

Double zones



Zone with feathered edge

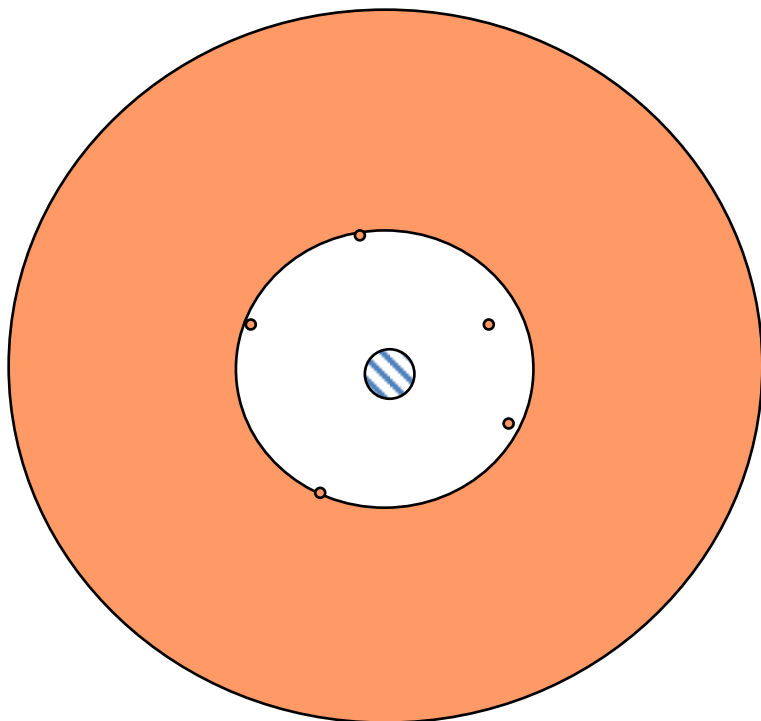


Measure the innermost zone

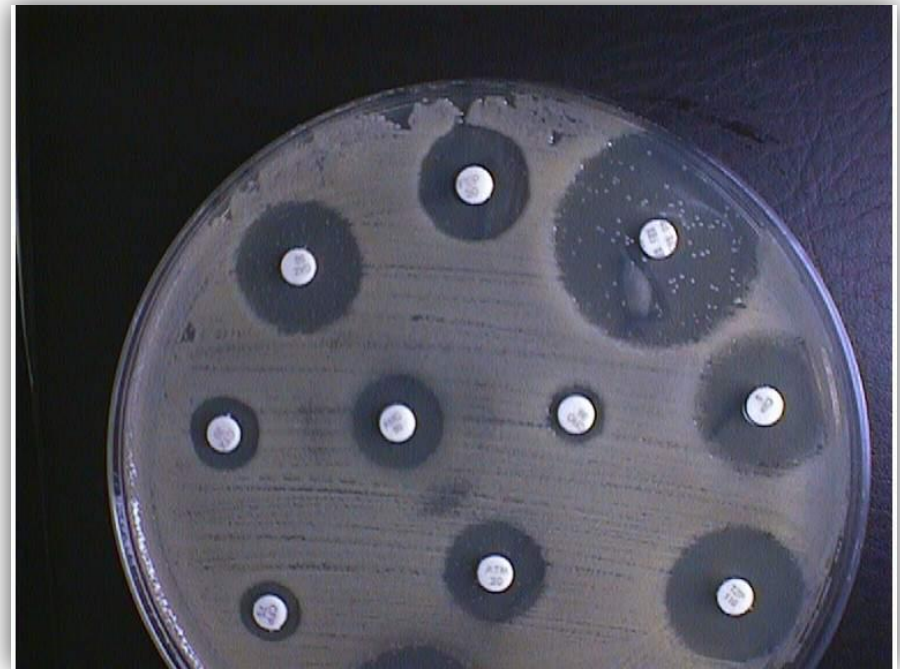
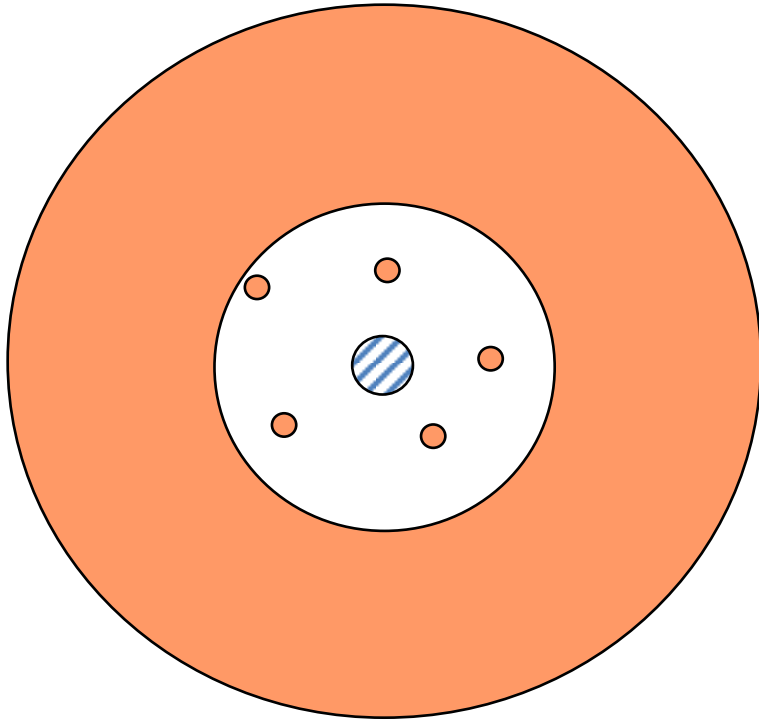


資料來源：Manual of Antimicrobial Susceptibility Testing. (2005). ASM

Neglect The Tiny Colonies Esp. around The Edge

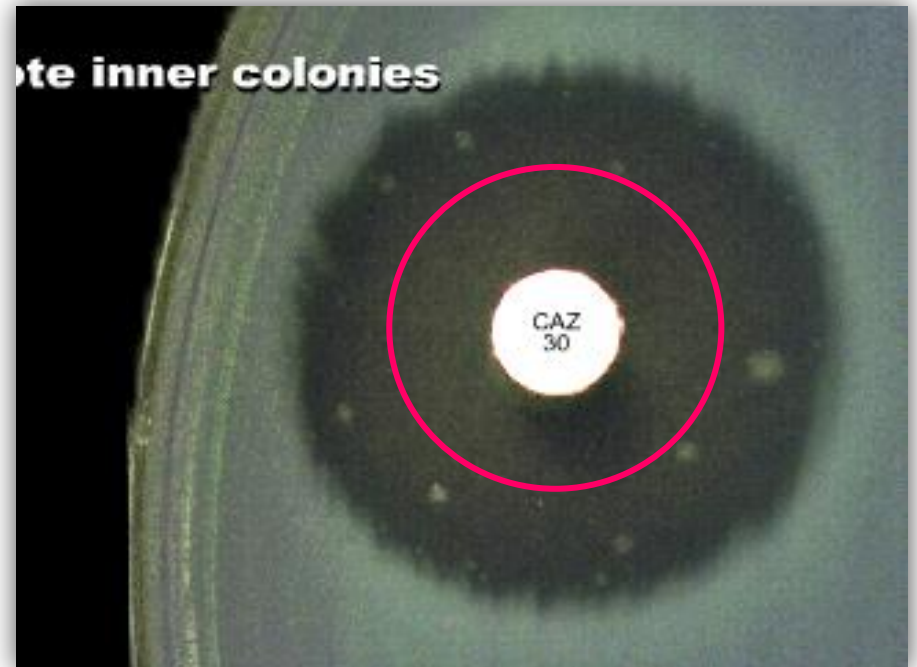
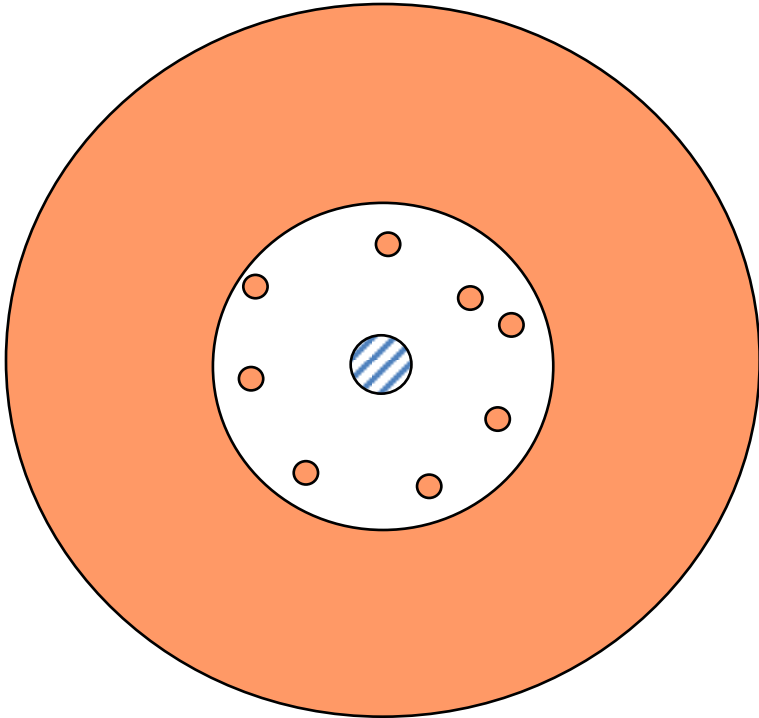


Large Colonies within The Clear Zone



- Contaminant ?
- Resistant variant ? Mixed heterogenous population

Zone with Inner Colonies

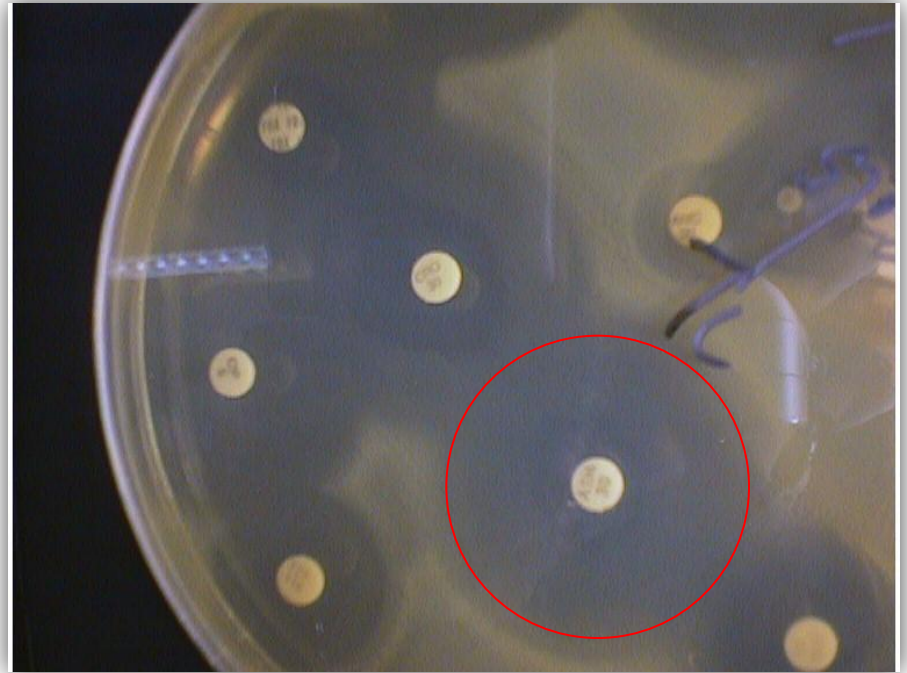
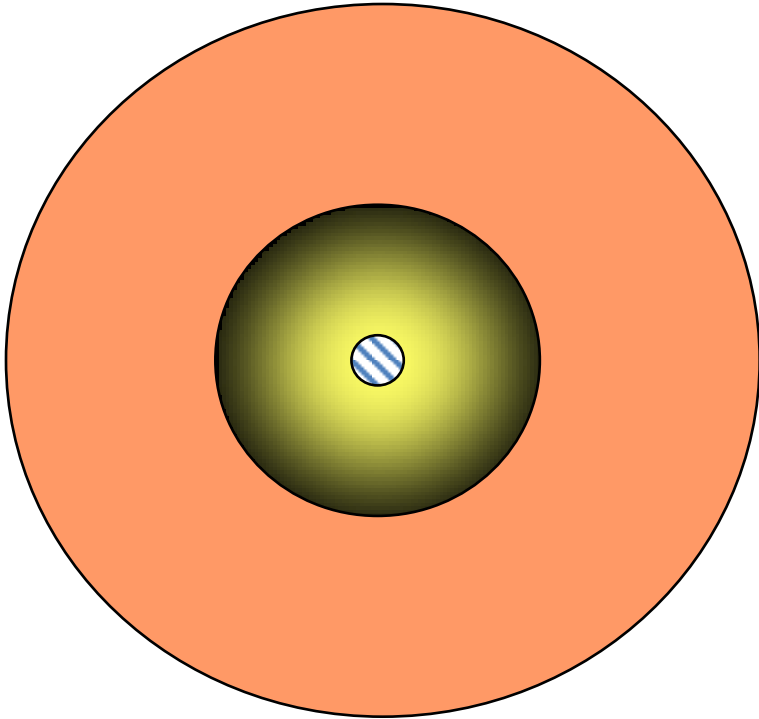


- Purity check
- Measure colony-free zone



資料來源：Manual of Antimicrobial Susceptibility Testing. (2005). ASM

Swarming of Proteus

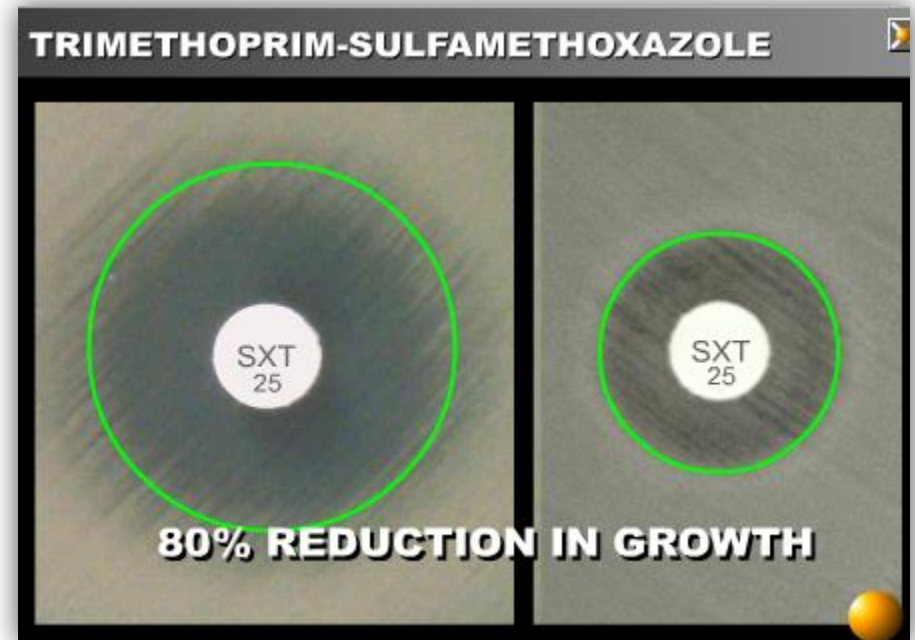
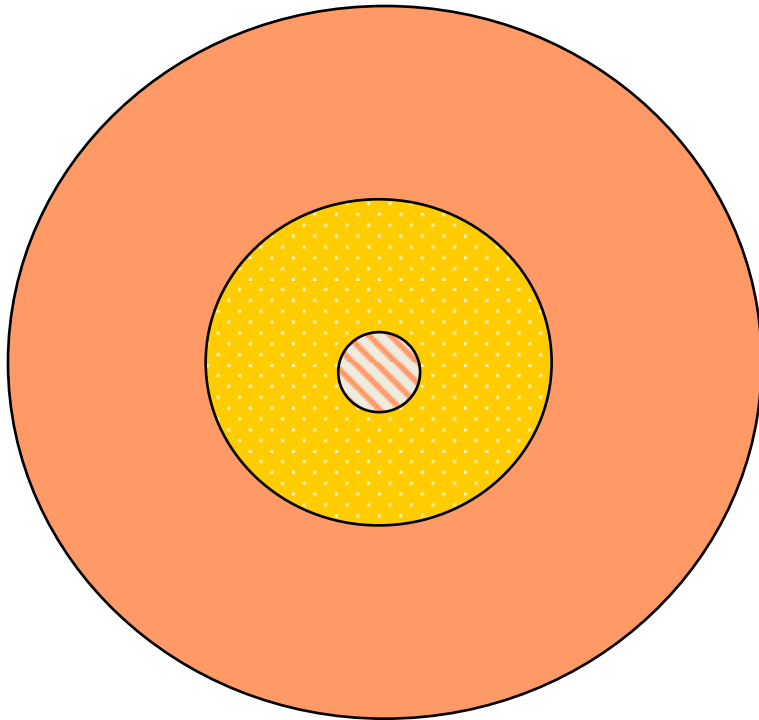


- Neglect the swarming area !!!



資料來源：Manual of Antimicrobial Susceptibility Testing. (2005). ASM

Zone with SXT



- Measure 80% reduction in growth



資料來源：Manual of Antimicrobial Susceptibility Testing. (2005). ASM



4. Reporting of AST



Reporting

- Organisms
- Specimens
- Special antibiogram
- Verification Guidelines



Reporting of Specimens

- Fecal isolates of *Salmonella* and *Shigella* spp.
 - Only routinely reported AM, a fluoroquinolone, and SXT
- Extraintestinal isolates of *Salmonella* spp.
 - Tested and reported chloramphenicol and a 3rd cepha.
- CSF
 - Don't report: only oral route agent, 1st and 2nd cepha., cephamycins, macrolides, tetracyclines, fluoroquinolone
- Urine tract
 - Not reported macrolides and chloramphenicol



Report of Special Antibiogram Do Not Report

- ESBL
 - All penicillins, cephalosporins, and aztreonam should be reported as resistant, **even if in vitro test results indicate susceptibility**
- MRSA
 - ALSO resistant to all penems, cephems, and other beta-lactams such as AMC, TZP, IPM



Intrinsic Resistance

Organisms	Natural Resistance Against
Anaerobic bacteria	Aminoglycosides
Aerobic bacteria	Metronidazole
Gram-positive bacteria	Aztreonam (a beta-lactam)
Gram-negative bacteria	Vancomycin
<i>Klebsiella</i> spp.	Ampicillin (a beta-lactam)
<i>Stenotrophomonas maltophilia</i>	Imipenem (a beta-lactam)
Lactobacilli and <i>Leuconostoc</i>	Vancomycin
<i>Pseudomonas aeruginosa</i>	SXT, tetracycline, or chloramphenicol
Enterococci	Aminoglycosides (except highlevel) All cephalosporins

※ Include CLSI M100-S23 appendix B – Intrinsic resistance - *Enterobacteriaceae*



Verification Guidelines(1/2)

- Verification—Relatedness of Cephalosporins
 - *Enterobacteriaceae*
 - 4th > 3rd > 2nd > 1st
 - *P. aeruginosa* (only 3rd and 4th are active)
 - cefepime = ceftazidime > cefoperazone > ceftizoxime = ceftriaxone = cefotaxime

※(> Means activity is greater than and = means activity is comparable to)



資料來源：Cavalieri., 2005. Manual of antimicrobial susceptibility testing. ASM



Verification Guidelines(2/2)

- Verification—Relatedness of Penicillins
 - *Enterobacteriaceae*
 - Piperacillin-tazobactam > piperacillin = mezlocillin > ticarcillin = carbenicillin > ampicillin
 - *P. aeruginosa*
 - Piperacillin-tazobactam = piperacillin > mezlocillin = ticarcillin > carbenicillin



資料來源：Cavalieri., 2005. Manual of antimicrobial susceptibility testing. ASM



Suggestions for Confirmation

- Category I
 - Not reported or only rarely reported to date
 - Repeat testing unless had isolate previously
- Category II
 - Uncommon in most institutions
 - Repeat testing
- Category III
 - May be common, but is generally considered of epidemiological concern



資料來源：CLSI M100-S23

Suggestions for Confirmation - GNB

Organism	Resistance phenotype detected	I	II
Any <i>Enterobacteriaceae</i>	Carbapenem – I or R		X
<i>Salmonella</i> and <i>Shigella</i>	Cephalosporin III – I or R		X
	Fluoroquinolone – I or R		
<i>A. baumannii</i>	Colistin/polymyxin – R		X
<i>P. aeruginosa</i>	Colistin/polymyxin – R		X
<i>S. maltophilia</i>	SXT – I or R		X
<i>H. influenzae</i>	Carbapenem – NS	X	
	Extended-spectrum cepha. – NS		
	Fluoroquinolone – NS		
	AMC – R		X
	AM – R and β -lactamase-negative		
<i>Neisseria gonorrhoeae</i>	Extended-spectrum cepha. – NS		X



資料來源：CLSI M100-S23 , modified

Suggestions for Confirmation - GPC(1/2)

Organism	Resistance phenotype detected	I	II
<i>Enterococcus</i> spp.	Daptomycin – NS, Linezolid – R		X
<i>Staphylococcus aureus</i>	Vancomycin MIC \geq 8 μ g/mL		X
	Daptomycin – NS		X
	Linezolid – R		
	Quinupristin-dalfopristin – I or R		
	Vancomycin MIC = 4 μ g/mL		
<i>Staphylococcus</i> , coagulase-negative	Daptomycin – NS		X
	Linezolid – R		
	Quinupristin-dalfopristin – I or R		
	Vancomycin – I or R		



資料來源：CLSI M100-S23 , modified

Suggestions for Confirmation - GPC(2/2)

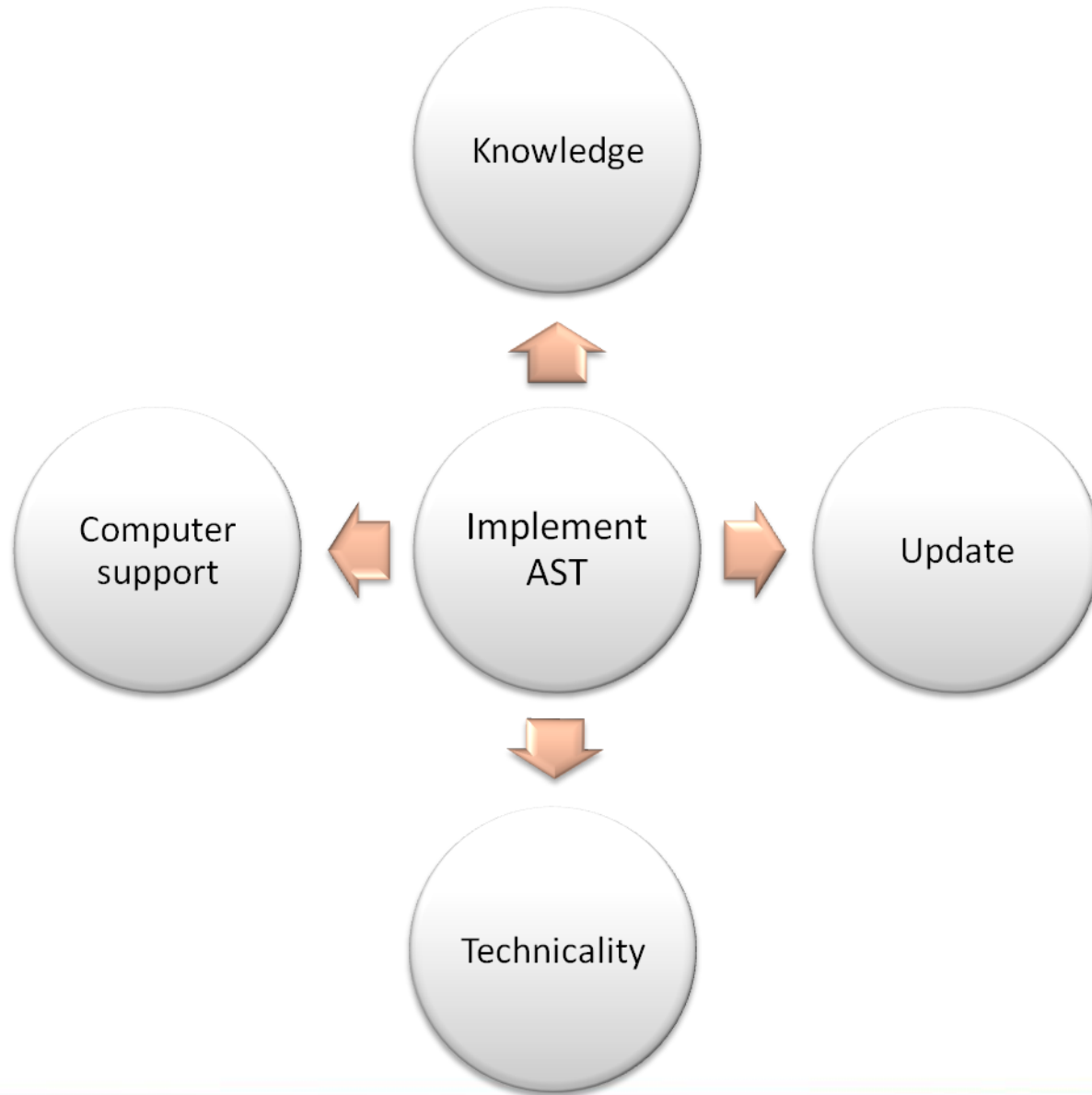
Organism	Resistance phenotype detected	I	II
<i>S. pneumoniae</i>	Linezolid – NS	X	
	Vancomycin – NS		
	Fluoroquinolone – I or R		X
	Imipenem or meropenem – I or R		
<i>Streptococcus</i> , β -hemolytic group	Ampicillin or penicillin – NS	X	
	Extended-spectrum cepha. – NS		
	Ertapenem or meropenem – NS		
	Linezolid – NS		
	Vancomycin – NS		
<i>Streptococcus</i> , viridans group	Daptomycin – NS	X	
	Ertapenem or meropenem – NS		
	Linezolid – NS		
	Vancomycin – NS		



資料來源：CLSI M100-S23 , modified



5. 結論





課程結束