Antimicrobial Susceptibility Testing (AST) in the era of Multi- Drug Resistant organisms

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Topics:

- Relevance of AST in managing infections along with importance of appropriate sampling
- Timelines for reporting
- Manual Vs automated options for AST
- Interpreting the AST results
- Case study- what does AST for MDR organisms look like?
- Recent updates on colistin and importance of genotyping

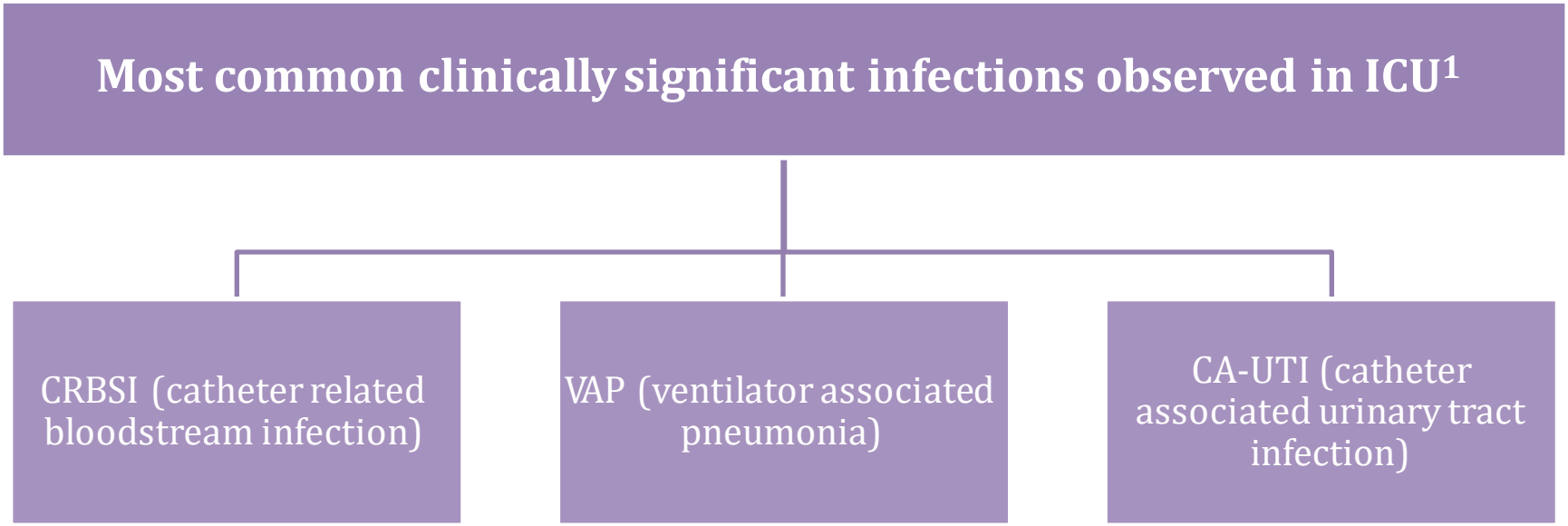
Relevance of AST in managing infections

The antimicrobial susceptibility testing helps to detect

- Possible drug resistance in common pathogens
- Susceptibility to chosen empirical antimicrobial agents for particular infections

Reller LB, Weinstein M, Jorgensen JH, Ferraro MJ. Antimicrobial susceptibility testing: a review of general principles and contemporary practices. Clinical infectious diseases. 2009;49(11):1749-55.

Importance of appropriate sampling



Sample collection, storage and transport is critical step in AST[2]

1. Burillo A, et al. Use of rapid diagnostic techniques in ICU patients with infections. BMC Infectious Diseases. 2014;14:593.
2. Hospital infection control guidelines. ICMR. Available at: https://www.ijmm.org/documents/Hospital_Infection_control_guidelines.pdf. Accessed on March 2021.

Importance of appropriate sampling

- Collection technique, storage and transport varies for each sample

	Blood	CSF	Nasopharyngeal swabs
Collection and inoculation	Inoculate immediately on the blood-culture media to prevent clotting in the syringe	If N. meningitidis is suspected to be the cause of the illness and a delay of several hours in processing specimens is anticipated, incubating the CSF at 35°C in a 5% CO_2 atmosphere may improve bacterial survival	Inoculate the swab on appropriate medium for direct plating
Storage	Blood culture bottle can be kept at room temperature (20°-25°C) for up to 8 hours. **Do not refrigerate**	**Do not refrigerate the CSF specimen** or expose it to temperature extremes	STGG can also be used for storage and transport (for a several hours at room temperature; for up to 8 weeks at -20°C; and, for at least 2 years at -70°C)
Transport	Sample should be receive by the laboratory within 12-18 hours for subculture. It should be protected from temperature extremes (<18°C or >37°C)	As soon as the CSF has been collected, it should be transported to the microbiology laboratory	Place the swab in STGG (Skim-milk tryptone glucose glycerol) transport medium for transportation to the laboratory
Recommendation	Ideally, the blood samples should be processed in a bacteriology laboratory within 2 hours of collection	Examine CSF sample within 1 hour of collection	Short-term storage of STGG is best at -70°C although a freezer at -20°C may also be used

Manual for the laboratory identification and antimicrobial susceptibility testing of bacterial pathogens of public health concern in the developing world. WHO. Appendix 3, 5. Available at: https://www.who.int/csr/resources/publications/drugresist/en/VAMRManual.pdf?ua=1. Accessed on March 2021.

Timelines for reporting

- Transport all specimens to the laboratory as soon as possible.
- Specimens must be sent to the microbiology laboratory in sterile, leak-proof containers in sealed plastic bags.
- If the specimens cannot be transported immediately to the laboratory, then urine, exudate, fluids, throat swab, ear and nasal swab, sputum, bronchoalveolar lavage, for culture and blood for serological testing can be refrigerated at 4 ºC (except for cold agglutination and complement detection), while blood culture bottles and CSF can be left at room temperature.

Hospital infection control guidelines. ICMR. Available at: https://www.ijmm.org/documents/Hospital_Infection_control_guidelines.pdf. Accessed on March 2021

Timelines for reporting

- Once the collected and samples are sent to laboratory for further evaluation, timelines for reporting result will depend on following steps

Standard inoculum preparation	• Choosing well-isolated colonies • Incubation for 18-24 hours
Broth microdilution	• Dilution of bacterial suspension (commonly 1:20) for MIC must occur within 15 minutes after making the standard inoculum
Inoculation and incubation	• Inoculation on suitable growth medium • Incubate for 18-24 hours
Reading the results	• Measure zone of inhibition or MIC • Interpretation of AST results

1. Bayot ML, Bragg BN. Antimicrobial Susceptibility Testing. [Updated 2020 Aug 5]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2020 Jan. Available from: https://www.ncbi.nlm.nih.gov/books/NBK539714/. Accessed on Feb 2021.
2. Manual for the laboratory identification and antimicrobial susceptibility testing of bacterial pathogens of public health concern in the developing world. WHO. Appendix 7. Available at: https://www.who.int/csr/resources/publications/drugresist/en/VAMRManual.pdf?ua=1. Accessed on March 2021.

Options available for AST

Commercial systems for AST can be divided into automated, semiautomated, and manual.

Automated testing system:

Consists of automated inoculation of MIC panels followed by computer-assisted incubation with reading, interpretation, and reporting functions that do not require manual intervention.

Semiautomated system:

Consists of manual or automated inoculation of panels and manual off-line incubation of panels.

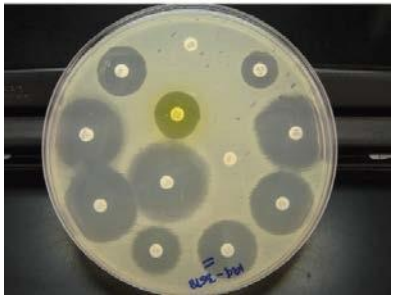
Each panel is then loaded into an automated reader, and computer-assisted reading and interpretive reporting of MICs is performed.

Manual AST panels:

Inoculated and incubated manually, read visually by laboratory personnel, and the results are either recorded by hand or manually entered into a computer for interpretation and reporting.

Evangelista AT, Karlowsky JA. Automated and Manual Systems for Antimicrobial Susceptibility Testing of Bacteria. Manual of Commercial Methods in Clinical Microbiology: International Edition. 2016 May 30:414-32.

Advantages & disadvantages of manual tests

	Advantages	Disadvantages
Antimicrobial gradient method 	It has intrinsic flexibility by being able to test the drugs the laboratory chooses	There are some systematic biases toward higher or lower MICs determined by the Etest
	This method is best suited to situations in which an MIC for only 1 or 2 drugs is needed	It can become expensive if more than a few drugs are tested
	Etest results have correlated well with MICs generated by broth or agar dilution methods	
Disk diffusion test 	Simple, practical and well-standardized method	Results are qualitative, accurate MIC can't be determined
	Results can be easily interpreted by all clinicians	Not all fastidious or slow growing bacteria can be accurately tested
	least costly of all susceptibility methods	

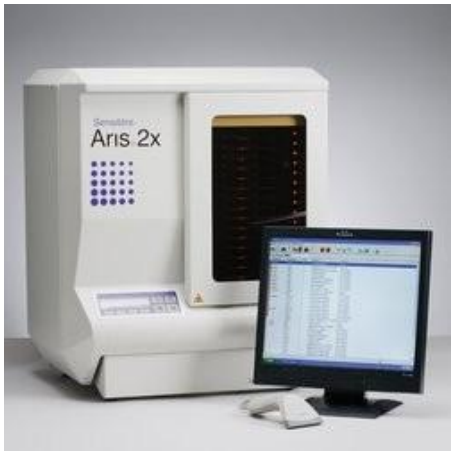
Reller LB, Weinstein M, Jorgensen JH, Ferraro MJ. Antimicrobial susceptibility testing: a review of general principles and contemporary practices. Clinical infectious diseases. 2009;49(11):1749-55.

Advantages and disadvantages of Automated instrument systems

Advantages	Disadvantages
Use of instrumentation can standardize the reading of end points and often	Expensive
Produce susceptibility test results in a shorter period than manual readings	Requires space for instrument
Better data management	Unable to detect some resistant phenotypes, e.g., vanB, vanC

1. Reller LB, Weinstein M, Jorgensen JH, Ferraro MJ. Antimicrobial susceptibility testing: a review of general principles and contemporary practices. Clinical infectious diseases. 2009;49(11):1749-55.
2. Schofield CB. Updating Antimicrobial Susceptibility Testing Methods. Clin Lab Sci. 2012;25(4):233.

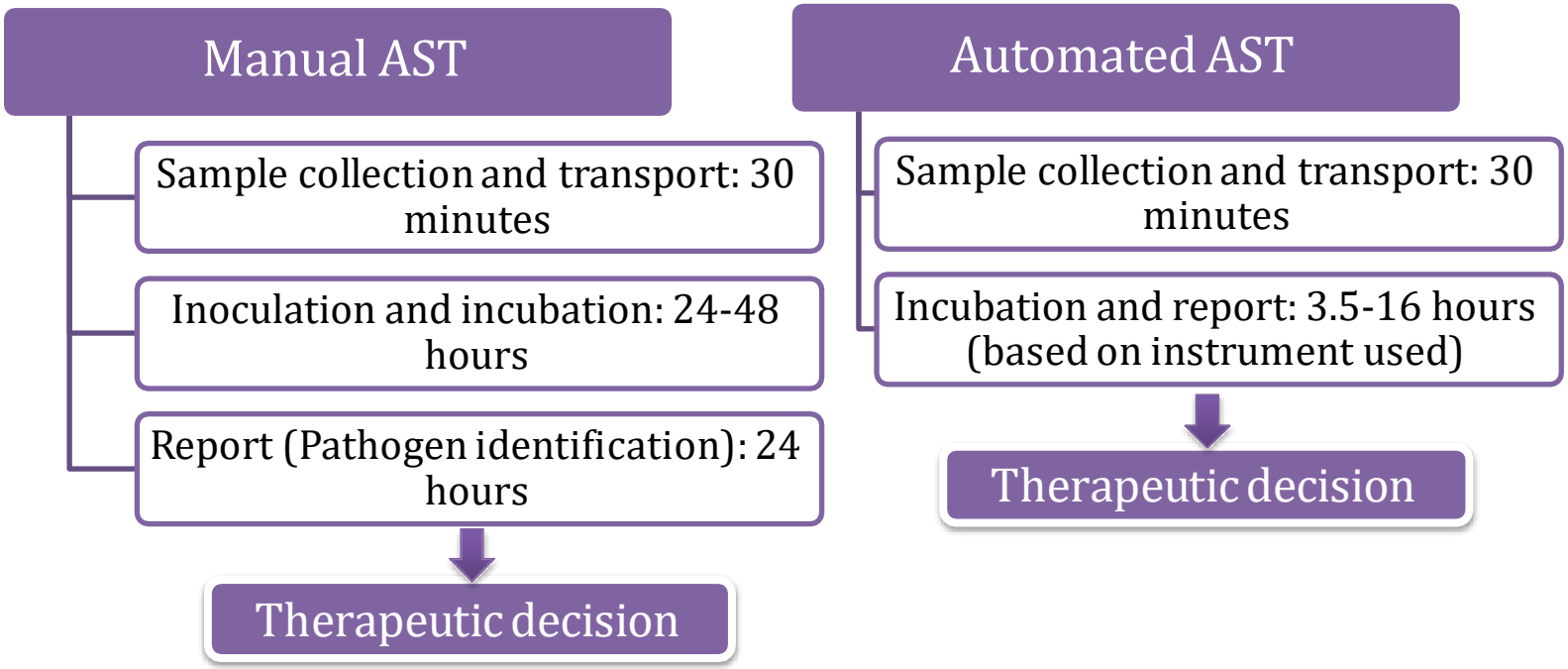
Automated instruments



1. MicroScan WalkAway *plus* System: https://www.beckmancoulter.com/products/microbiology/microscan-walkaway-plus-system Accessed on 2nd February 2020.
2. Reller LB, Weinstein M, Jorgensen JH, Ferraro MJ. Antimicrobial susceptibility testing: a review of general principles and contemporary practices. Clinical infectious diseases. 2009;49(11):1749-55.
3. The Vitek 2 System: https://www.biomerieux-usa.com/vitek-2 Accessed on 2nd February 2020.

Manual vs. automated AST

In case of hospital acquired infection, any delay in antibiotic treatment will worsen the prognosis and may also increase the mortality rate



With the use of Automated AST method, one can get report on the same working day; that can help clinicians to start early treatment

1. Burillo A, et al. Use of rapid diagnostic techniques in ICU patients with infections. BMC Infectious Diseases. 2014;14:593.
2. Schofield CB. Updating Antimicrobial Susceptibility Testing Methods. Clin Lab Sci. 2012;25(4):233.

Interpreting the AST results

- Optimal interpretation of MICs requires knowledge of the pharmacokinetics of the drug in humans, and information on the likely success of a particular drug in eradicating bacteria at various body sites
- Common terminologies used are,
  - **Susceptible (S)**: indicates that the patient's organism should respond to therapy with that antibiotic using the dosage recommended normally for that type of infection and species
  - **Resistant (R)**: indicates that an organism should not be inhibited by the concentrations of the antibiotic achieved with the dosages normally used with that drug
  - **Intermediate (I)**: indicates that clinical response is likely to be less than with a susceptible strain.

Jorgensen JH, et al. Antimicrobial Susceptibility Testing: A Review of General Principles and Contemporary Practices. Clinical Infectious Diseases. 2009;49(11):1749-1755.

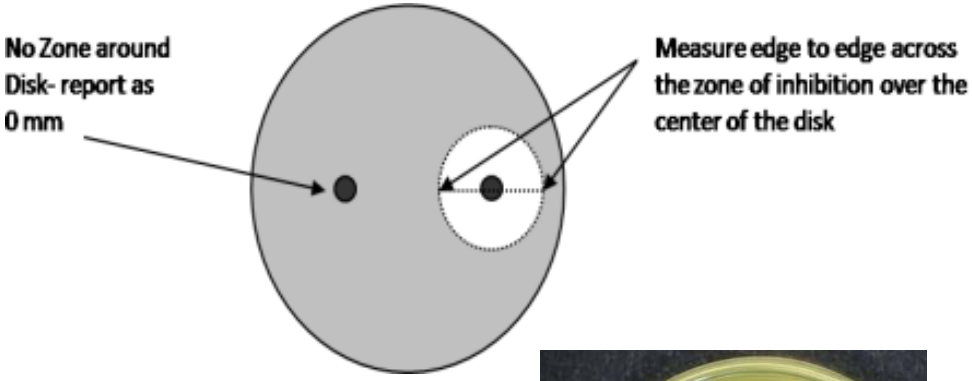
- **MIC:** Minimum concentration of an antibiotic needed to inhibit visible growth of a single isolate of an organism. Important for definitive treatment of an individual patient.
- **Breakpoint:** Discriminatory concentrations used in the interpretation of results of susceptibility testing to define isolates as susceptible, intermediate, or resistant (determined by various organizations - FDA, CLSI, EUCAST)

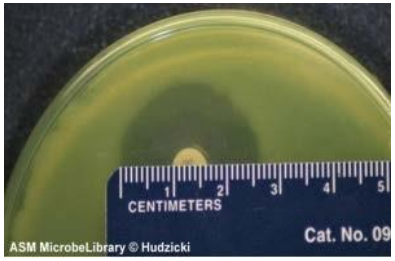
Updates on antimicrobial stewardship. Available at: https://www.sahealth.sa.gov.au/wps/wcm/connect/6b86eddf-3bd4-4dfc-ada4-2ccc95244318/MICS_Breakpoints_AMS_Updates_Issue1__2018_SAAGARversion.pdf?MOD=AJPERES&CACHEID=ROOTWORKSPACE-6b86eddf-3bd4-4dfc-ada4-2ccc95244318-niQTcCC.

Disk diffusion method: Interpretation of the results

The results of the disk diffusion test are “qualitative,” in that a category of susceptibility (ie, susceptible, intermediate, or resistant) is derived from the test rather than an MIC.

The diameter of the zone is related to the susceptibility of the isolate and to the diffusion rate of the drug through the agar medium.





The zone diameters of each drug are interpreted using the criteria published by the Clinical and Laboratory Standards Institute (CLSI) or those included in the US FDA– approved product inserts for the disks.

Reller LB, Weinstein M, Jorgensen JH, Ferraro MJ. Antimicrobial susceptibility testing: a review of general principles and contemporary practices. Clinical infectious diseases. 2009;49(11):1749-55.

Zone Diameter and Minimal Inhibitory Concentration Interpretive Standards for *Enterobacteriaceae*

Test/ report group	Antimicrobial agent	Disk content	Zone Diameter Interpretive Criteria (nearest whole mm)				MIC Interpretive Criteria (µg/mL)			
			S	SDD	I	R	S	SDD	I	R
β-LACTAM/β-LACTAMASE INHIBITOR COMBINATIONS (Pseudomonas aeruginosa)										
B	Piperacillin-tazobactam	100/10 µg	≥ 21		15–20	≤ 14	≤ 16/4		32/4-64/4	≥ 128/4
O	Ticarcillin-clavulanate	75/10 µg	≥ 24		16–23	≤ 15	≤ 16/2		32/2-64/2	≥ 128/2
β-LACTAM/β-LACTAMASE INHIBITOR COMBINATIONS (Enterobacteriaceae)										
B B B B	Amoxicillin-clavulanate Ampicillin-sulbactam Piperacillin-tazobactam Ticarcillin-clavulanate	20/10 µg 10/10 µg 100/10 µg 75/10 µg	≥ 18 ≥ 15 ≥ 21 ≥ 20		14-17 12-14 18-20 15-19	≤ 13 ≤ 11 ≤ 17 ≤ 14	≤ 8/4 ≤ 8 /4 ≤ 16/4 ≤ 16/2		16/8 16/8 32/4-64/4 32/2-64/2	≥ 32 /16 ≥ 32 /16 ≥ 128/4 ≥ 128/2
A	Cefazolin	30 µg	≥ 23		20-22	≤ 19	≤ 2		4	≥ 8
C	Ceftaroline	30 µg	≥ 23		20-22	≤ 19	≤ 0.5		1	≥ 2

M100-S25 Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fifth Informational Supplement. Clinical and Laboratory Standards Institute antimicrobial susceptibility testing standards. 30th edition. 2015;35(3) page 45 & 53.

Importance of Genotyping

- Genotypic AST are effective direct methods that eliminate tedious bacterial cultures, long incubation, chances of contamination, and the spreading of deadly infections.
- PCR, DNA microarray and DNA chips, and loop-mediated isothermal amplification (LAMP) are some of the genotypic techniques for the detection of antibiotic resistance.
- Rapid detection of resistance gene within few hours that helps in making decision to use more specific antimicrobial agent rather than broad spectrum antimicrobial agent.

Khan ZA, Siddiqui MF, Park S. Current and emerging methods of antibiotic susceptibility testing. Diagnostics. 2019;9(2):49.
Bard JD, et al. Why Can't We Just Use PCR? The Role of Genotypic versus Phenotypic Testing for Antimicrobial Resistance Testing. Clin Microbiol Newsl. 2018;40(11):87-95.

Case study- what does AST for MDR organisms look like?

- **Patient name:** Mr. XYZ
- **Age:** 49 years
- **Medical history:** The patient was admitted into a XYZ nursing home with complaints of fever, chills and persistent coughing.
- Patient had been put on following medications: Amoxycillin (500mg) + Clavulanic Acid (125mg), Azithromycin 250mg and Paracetamol 650mg.
- The patient started complaining of blood in mucus, severe chest pain and even shortness of breath. He has been referred to ABC multispecialty hospital for advanced care. Headmitted to ICU ward and put on ventilator.

- **Diagnostics test:** Trans-Tracheal aspiration was performed to collect sample from the lungs and lower airways for antimicrobial susceptibility testing using disk diffusion method.
- Collected specimen was containing *Klebsiella pneumoniae* iidentified by their morphology and biochemical characteristics. In Gram staining, Gram-negative, short, blunt rods were seen.
- The antimicrobial sensitivity of the test strains of Gram Negative antibacterial drugs was done using the KirbyBauer disk diffusion method.
- The commercial available antibiotic discs used for the study were Meropenem, Imipenem, Piperacillin-tazobactam, Ceftriaxone, Levofloxacin, Colistin, Ampicillin, Gentamycin, Ceftazidime/avibactam, Ciprofloxacin and Cefotaxime.
- Prepared disks were incubated at 37°C for 24 hours and than the diameters of inhibition zone were measured using a metric ruler to determine MIC

Susceptibility testing report

Date: 16-02-2021

Name: Mr. JN Nair
Age: 49 years
Gender: Male

Antibiotic	MIC	
	µg/ml	Interpretation
Meropenem	8	R
Imipenem	8	R
Piperacillin-tazobactam	128	R
Ceftriaxone	8	R
Levofloxacin	16	R
Colistin	**1**	**S**
Ampicillin	64	R
Gentamycin	16	R
Ceftazidime/avibactam	**2**	**S**
Ciprofloxacin	4	R
Cefotaxime	4	R

Report assessment

- Lab reports indicate that patient is infected with *Klebsiella pneumoniae* (*K. pneumoniae*) which is resistant to several antibiotics.
- Reports indicate that the bacteria is sensitive to colistin and it is also susceptible to Ceftazidime/avibactam

Current issues with colistin

- Rise in carbapenem resistance leads to increased usage of colistin, which eventuallyincreases colistin resistance.
- Handling of colistin challenges involves both laboratory as well as therapeutic management.
- Therapeutic challenges such as, high nephrotoxicity and neurotoxicity; promotion of resistance during sub-optimal dosage; lack of universal dosing in critically ill patients; narrow therapeutic window and low mutant prevention concentration.
- Apart from these colistin has poor penetration into the lung parenchyma that questions it's efficacy in the treatment of VAP

VAP: ventilator associated pneumonia

Veeraraghavan B, et al. Colistin-sparing approaches with newer antimicrobials to treat carbapenem-resistant organisms: Current evidence and future prospects.. Indian J Med Microbiol. 2019;37:72-90.
Cisneros JM, et al. Colistin versus meropenem in the empirical treatment of ventilator-associated pneumonia (Magic Bullet study): an investigator-driven, open-label, randomized, noninferiority controlled trial. Crit Care. 2019;23(1):383.

Recent updates on colistin and importance of genotyping

- CLSI has removed the susceptibility breakpoints for colistin against *Enterobacterales, P. aeruginosa and Acinetobacter spp*.
- There are numerous issues related with colistin use and hence identification of colistin resistance mechanism by genotyping becomes crucial.
- This can help clinicians to choose appropriate antimicrobial agent.

Satlin MJ, et al. Clinical and Laboratory Standards Institute and European Committee on Antimicrobial Susceptibility Testing Position Statements on Polymyxin B and Colistin Clinical Breakpoints. Clin Infect Dis. 2020;71(9):e523-e529.
Veeraraghavan B, et al. Colistin-sparing approaches with newer antimicrobials to treat carbapenem-resistant organisms: Current evidence and future prospects.. Indian J Med Microbiol. 2019;37:72-90.

Actions taken based on report

- Avibactam (AVI) is a potent, novel diazabicyclooctane β-lactamase inhibitor with *in vitro* activity against classes A, C and some class D β-lactamases. When used in combination with ceftazidime (CAZ), AVI restores the activity of CAZ against Gram-negative organisms producing these carbapenemases, as well as any co-carried ESBLs
- Taking in consideration of issues related to colistin in ICU setting and positive data of avibactam/ceftazidime
  - This patient started on Ceftazidime/avibactam therapy

Yang et al. BMC Microbiology. 2019;19:240.

Thank You..