

Emerging resistance mechanisms and their effect on reading antibiograms

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Empirical Therapy

- In the past, many infections could be successfully treated according to the clinician's experience and knowledge
- Susceptibility could be reliably **predicted**
- Prudent microbial use dictates that far more use should be made of antibiograms to select the correct treatment

Worldwide antibiotic resistance

- Concern over multiple antimicrobial resistant pathogenic bacteria, both in animals and man
- OIE, FAO and WHO
- Resistance now so common, that far more cases require antibiograms
- **Accurate** diagnostic laboratory results are critically important, to help control the spread of resistance

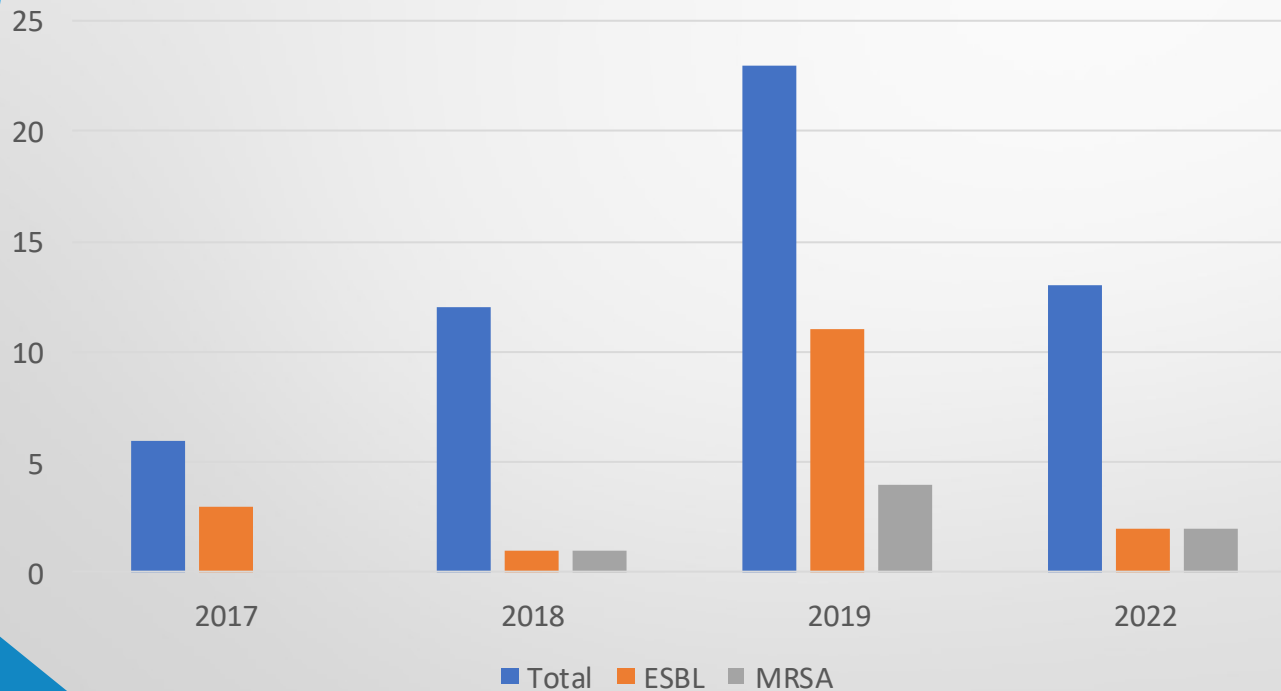
MDR and XDR

- MDR – Multiple Drug Resistant defined as resistant to 3 or more classes of antibiotics
- XDR – Extreme Drug Resistant defined as resistant to all drugs tested
- MRSA [Methicillin Resistant *Staphylococcus*] and ESBL [Extended Spectrum Beta Lactamase] are often MDR or XDR

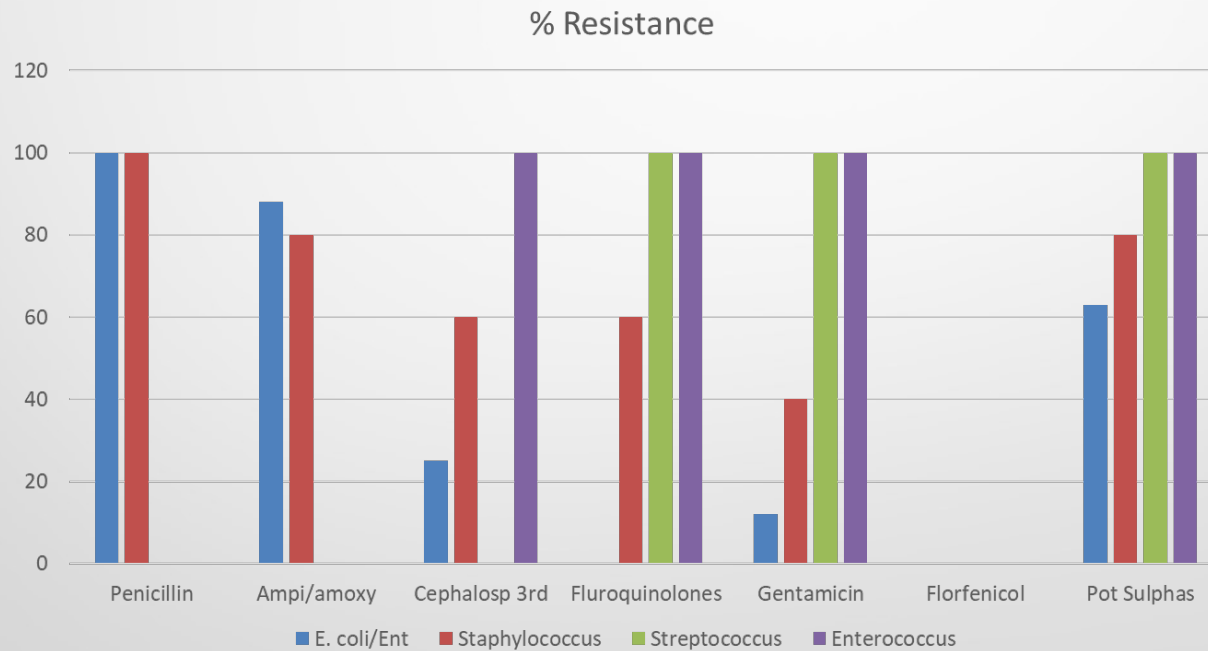
ESBL and MRSA in post op equine infections

2017/8/9 & 2022

Increase 2017-19; Post Covid drop



Wound infections horses Jan-Sept 2022



Interpreting antibiograms is becoming more complicated

- Far more information on antibiotic action is available resulting in better correlation between lab result and patient outcome
- New resistance mechanisms – genetic tests are the gold standard, but are expensive, time consuming and mostly not available as diagnostic tests
- Some resistance mechanisms are now rapidly detectable in ordinary laboratories. Not all are valid for animal pathogens.

Ensuring the correct outcome

- Requires standardized methods
- Harmonization of test parameters
- Accurate and reproducible data
- Appropriate antibiotics
- Additional specific antimicrobial tests
- Genome sequencing – in the future

Antimicrobial susceptibility testing guidelines

- Human isolates

CLSI - USA

EUCAST – Europe

Veterinary guidelines are being developed but they are only in their infancy at present

VetCAST and **CLSI** Veterinary

Natural resistance

- *E. coli* to penicillin
- Aerobes to metronidazole
- *Streptococcus* to aminoglycosides
- Interpretive rules address natural resistance **as well as** newly discovered resistance mechanisms

EUCAST expert rules

- Clinical **and** microbiological evidence used for new interpretation of AMS testing
- If there is a resistance mechanism present, the antibiogram should be changed to resistant
- Any modifications must follow an expert rule
- VetCAST will set rules for animal isolates
- Rules are per bacterium. There are different rules for *E. coli*, *Staphylococcus*, *Streptococcus*, etc.

Expert rule on misleading results for *Salmonella* and *Enterococcus*

The following antibiotic/organism combinations may appear sensitive, but are not clinically effective, and must not be reported as susceptible

Organism	Do not report as susceptible
Salmonella	1 st & 2 nd gen cephalosporins, cephamycins, aminoglycosides
Enterococcus	Aminoglycosides, cephalosporins, clindamycin, potentiated sulphas

Other expert rules

- *Klebsiella* is resistant to ampicillin
- *Streptococcus* is resistant to aminoglycosides, tetracyclines and fluroquinolones
- Fluroquinolones are not effective in anaerobic conditions
- Clavulanic acid is a suicide inhibitor. If the pathogen is resistant to amoxycillin but sensitive to amoxiclav, and is present in large numbers, the clavulanic acid is likely to be depleted rapidly.

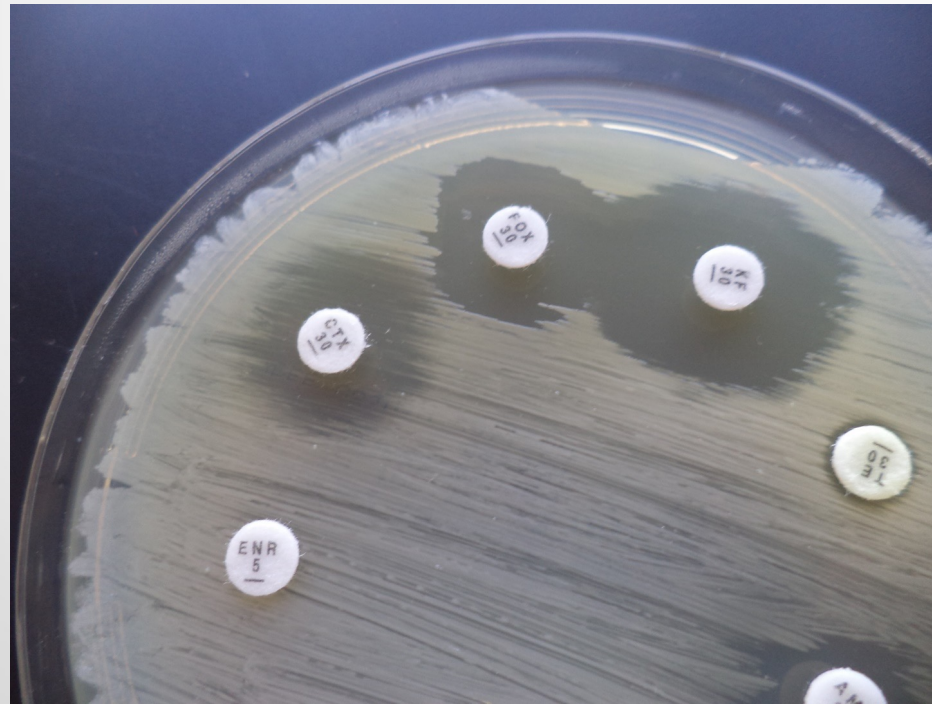


Duty of the Laboratory

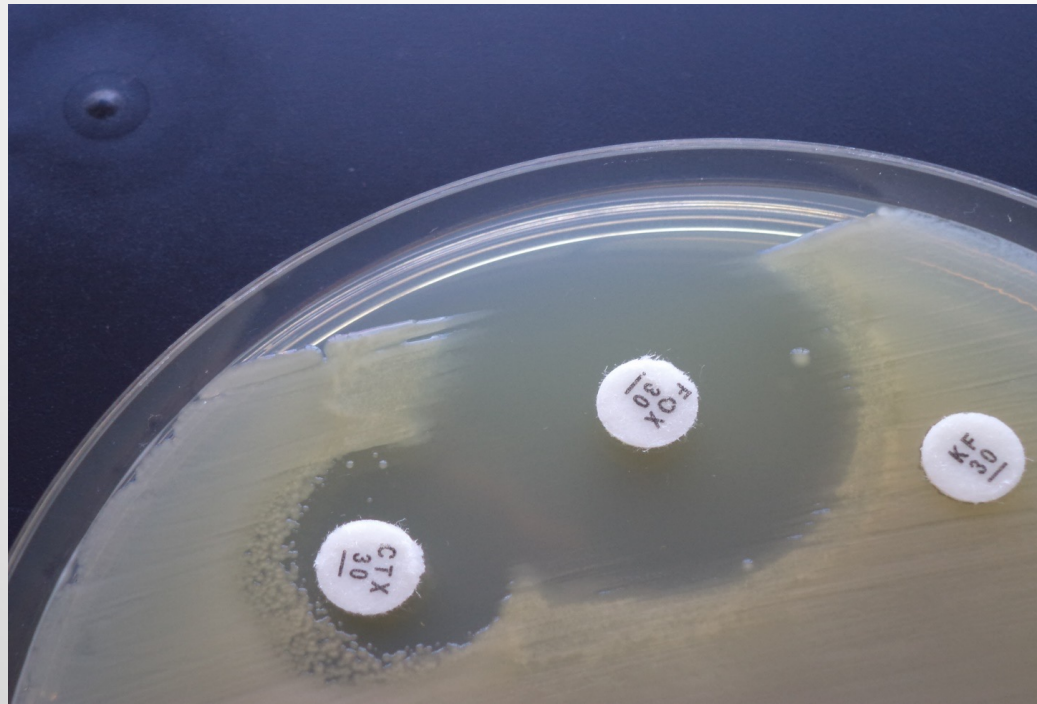
Warning signs of ESBL and MRSA

- Irregular zones around discs
- Patterns of resistance that don't fit in – the usual sequence of penicillin resistance is penicillin, then ampicillin or amoxycillin, and lastly ampicillin with clavulanic acid.
- Cephalosporins usually follow the 1 – 2 – 3 generation pattern as well

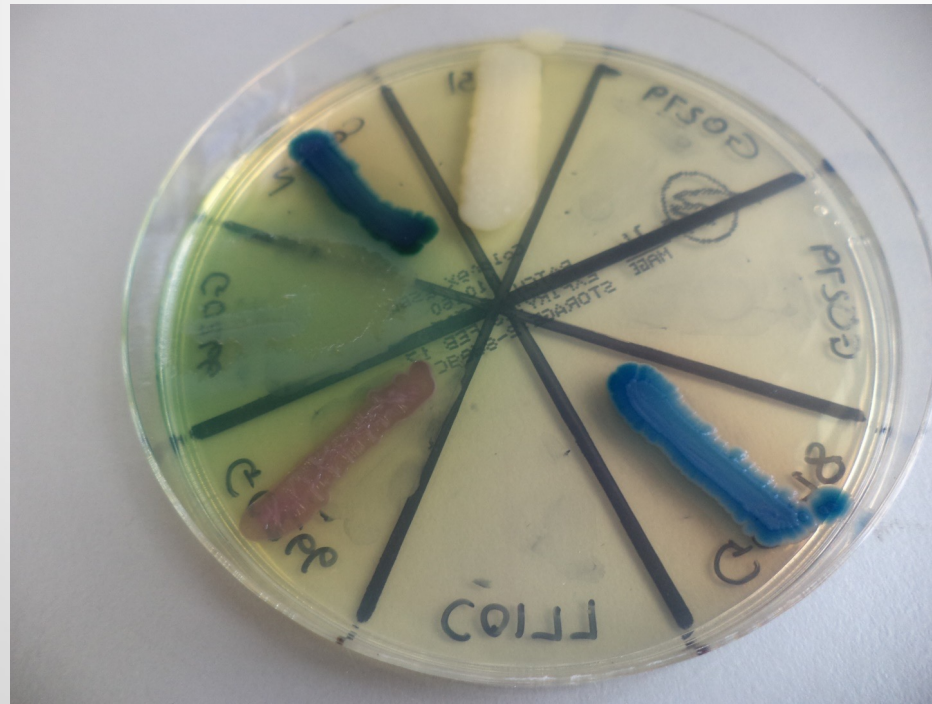
Irregular zone and pattern



Atypical pattern – only second generation cephalosporins sensitive, 1st and 3rd resistant



ESBL detection using chromogenic agar



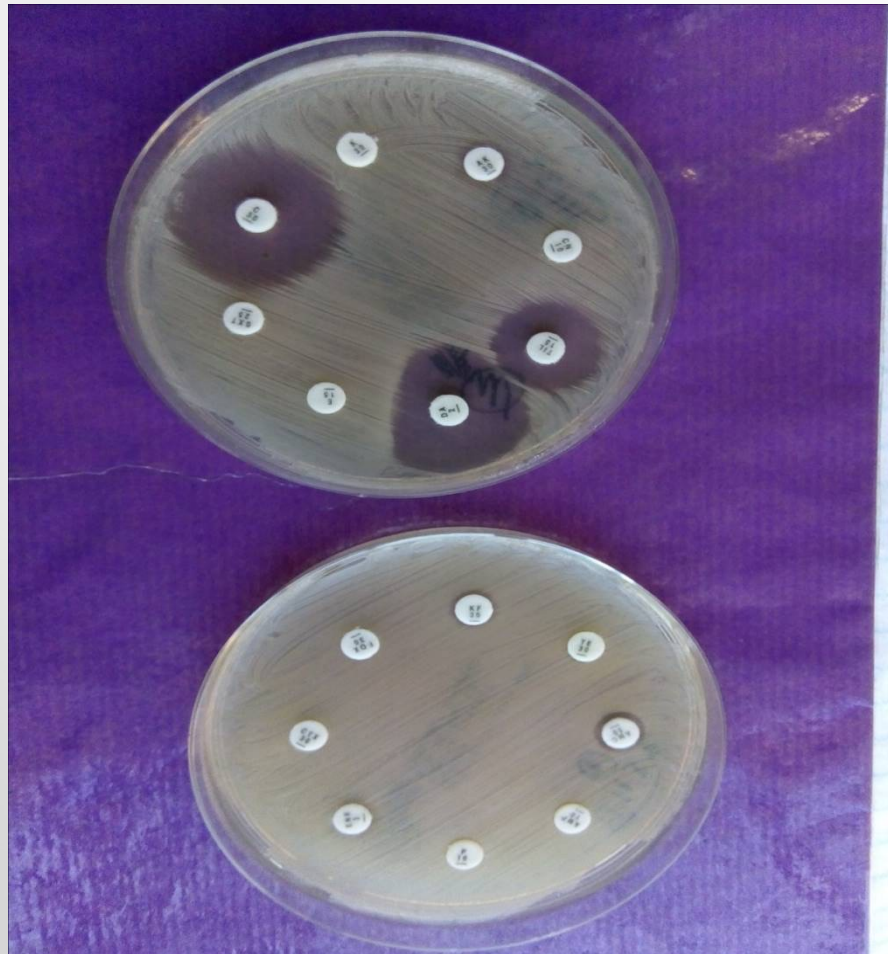
Induced resistance

- Switched off genes
- Related to clinical failures
- The use of the antibiotic selects for resistant mutants
- For macrolides, the D shape zone indicates inducible resistance to lincomycin or clindamycin

D-shape inducible resistance



D-zone for *Staphylococcus* often indicates multiple drug resistance





What you can do

Interpreting antibiogram results

Valid Extrapolations for inferring susceptibility

Antibiotic result	Also valid for
Ampicillin	Amoxycillin
Cephalosporin 1 st generation	All in first generation
Cephalosporin 2 nd generation	All in second generation
Erythromycin [class representative]	Azithromycin and Clarithromycin
Clindamycin	Lincomycin
Tetracycline	Doxycycline [usually]
Enrofloxacin	All available veterinary fluoroquinolones

Warning sign of errors

Antibiotic	Warning of possible error	Likely to be correct
Penicillin	R	R
Ampicillin	S	R
AmoxyClav	R	S
First generation cephalosporin	S	R
Second generation cephalosporin	S	S
Third and 4 th generation cephalosporin	R	S

Stepwise resistance for aminoglycosides

Aminoglycoside	Sensitive or Resistant
Amikacin	S
Tobramycin	S
Gentamycin	S
Neomycin	R
Kanamycin	R
Streptomycin	R

Multibacterial infections

- Invariably a Gram positive and a Gram negative together
- Antibigrams rarely yield a single antibiotic recommendation
- Use synergism and avoid antagonism

Synergism

- Penicillin group with aminoglycosides - penicillin allows more aminoglycoside to be taken up
- Rifampin with erythromycin group
- Sulphas with polypeptides

Sensitive but poor outcome

- Did the antibiotic reach the tissue and bug?
- Does the immune system work?
- Are there anaerobes or fungi present as well, which were not cultured for?
- Are one of the new resistance mechanisms present?

Summary

- The new interpretative rules are complicated and organism specific
- Request antibiograms as soon as empiric selection fails
- Overuse of antibiotics can select for plasmids carrying resistance for many antibiotics
- Explore other options such as disinfectants