AB susceptibility testing in support to responsible AB use in veterinary medicine: current challenges

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Best Practice to reduce Antibiotic Resistance

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New Royal Decree regulating the use of drugs by Vets and Animal owners (MB/BS 29-07-2016)
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- **DRUG DEPOSIT**: Admin. management, Registering, Reporting, Archiving, Transfering
- **DRUG DELIVERY**: Registering, Prescription to FOOD / NON-FOOD producing animals
- **SPECIAL MEASURES** (not for HORSES nor for INTRA-HUDDER delivery of critical ABs)

  - Use of critical ABs (FQ1,2,3 + CEPH 3,4) is **forbidden** unless a number of criteria are fulfilled. These criteria include:
    - lab isolation of the disease-causing bacterium,
    - demonstration of its resistance to $\geq 5$ non-critical AB
    - demonstration of its susceptibility to $\geq 1$ critical AB
  - If lab analyses are not feasible (sampling or culture or susceptibility testing is difficult/impossible), literature data supporting the sole use of critical ABs must be provided.
New Royal Decree regulating the use of drugs by Vets and Animal owners (MB/BS 29-07-2016)

- SPECIAL MEASURES (continued)
  - In exceptional (emergency) cases, a critical AB may be administered to a single animal under the Vet’s own responsibility:
    - life-threatening stage of the disease or
    - risk of irreversible sequelae.

Lab analyses remain due and treatment shall be adapted upon results’ delivery.
Use of critical AB?

- Very disuasive regulation
- Are situations where critical AB use is permitted realistic?
- Will other « critical » or « last-resort » molecules later be merged to the list? (Colistine, Pleuromutilins, …)
About *E. coli* resistance to ≥ 5 non-critical ABs ... 

- EU standard µ-dilution plate: 2016 MIC data

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- In a panel of 1,073 indicator *E. coli* strains isolated non-specifically < pigs, cattle and poultry, 12 (**1.1%**) do resist to ≥ 5 non-critical ABs and are sensitive to either FQ or CEPHA or both.

- In a panel of 483 ESBL-producing indicator *E. coli* strains isolated < pigs, cattle and poultry, 34 (**7%**) do resist to ≥ 5 non-critical ABs from which just 4 (**0.8%**) are sensitive to FQ
Use of critical AB?

- Very disuasive regulation
- Are situations where critical AB use is permitted realistic?
- Will other «critical» or «last-resort» molecules later be merged to the list? (Colistine, Pleuromutilins, …)

=> Time to **Promote AB Susceptibility Testing (AST)** as a support to AB treatments?!
- When is it relevant?
- What is needed?
- What is the future?
About the lab isolation of a disease-causing bacterium...

○ FROM THE VETERINARY SIDE: « GVP ! »
  - Importance of the anamnesis / diagnostic
  - Relevance & Quality of the samples sent to the laboratory
  - Relevance of the requested analysis

SHARE DOUBTS ! DISCUSS ISSUES !
ADAPT SAMPLING !
REFINE / DOCUMENT DIAGNOSTIC !

○ FROM THE LABORATORY SIDE: « GLP ! »
  - Tentative isolation of the disease-causing bacterium
  - Identification of the pathogen + confirmation of the pathogenic potential if unsure
  - Antibiotic Susceptibility testing

Examples of « BVP / BLP »:
- Please conduct an AST on this swab ...
- Pullorose suspected, please confirm with the provided faeces ...

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Laboratory steps

- Tentative isolation of the disease-causing bacterium and relevance of subsequent AST

  - Highly specific enrichment / isolation media available (e.g. mobile *Salmonellae*, sporulating *Bacilli* / *Clostridia* ...) => isolation most probable, AST relevant
  - Semi- or Non-specific enrichment / isolation media available (*Streptococci*, *Pasteurellae*, *Salmonella Gallinarum* ...) => isolation probable, AST relevant

  - Enrichment / Isolation media available but slow or cumbersome growth (*Mycobacteria*, *Mycoplasmae*, *Leptospira*, *Brachyspira*...) => isolation hypothetical (expert labs only), AST not relevant

  - Bacterium is NOT culturable in free-living form (*Chlamydia*, *Rickettsia*, *Coxiella*, ...) => isolation highly challenging, AST not relevant
Laboratory steps

- How to deal with non-culturable bacteria?
  - Routine colony identification methods not relevant (MALDI-TOF MS), traditional AST impossible
  - Molecular (PCR) testing to rule in / rule out suspicion (more expensive)
  - Serological testing to rule in / rule out suspicion (requires additional blood sampling + seroconversion)
  - => Recommended (Antibiotic?) treatment based on Best Practice, Literature data, up-to-date Formularium
  - Awaited new methodologies for routine use in the Veterinary Bacteriology lab: Whole Genome Sequencing of bacteriological colonies, Sample / Metagenome / Microbiome Sequencing, AB resistance genes ID or screening (DNA hybridization micro-arrays)
Laboratory issues (1/4)

- The disease-causing bacterium is present and culturable but ...
  - hides within a flora of identical commensals (e.g. *E.coli*)

**DIAGNOSTIC OF ANIMAL COLIBACILLOSIS**

⇒ evidence for pathogenicity (*E.coli*) signatures needed (serotype, virulence factors, ...)
⇒ Molecular tests (PCR, hybridization µarrays)
⇒ Analyze bacterial pools

Drawbacks: analysis cost, need to analyze > 1 colony, delay
Laboratory issues (2/4)

- The disease-causing bacterium is present and culturable but ...
  - its presence is not necessarily a disease signature (opportunistic)

**DIAGNOSTIC OF OPPORTUNISTIC INFECTIONS**

⇒ link bacteriological finding with animal condition
⇒ check for records / symptoms of primary infections

Drawbacks: delay, risk of disease misidentification
Laboratory issues (3/4)

- The disease-causing bacterium is present and culturable but ...
  
  - is mixed with or inhibited by a bunch of other organisms (« polybacterial »)

  ⇒ Improve lab enrichment / (re-)isolation methods if possible
  ⇒ Shotgun identification of all colonies (MALDI-TOF)
  ⇒ Molecular detection of suspected pathogen(s) on pooled colonies / unenriched sample

Drawbacks: delay, analysis cost
The disease-causing bacterium is present and culturable but ...

- is present in very low quantities

⇒ Optimize lab enrichment / (re-)isolation methods if possible
⇒ Molecular detection of suspected pathogen(s) on unenriched sample

Drawbacks: delay, analysis cost
Conclusions

Use of Critical AB in animal productions strongly discouraged!

- very strict (unrealistic?) criteria (when AST is feasible)
- possibly more critical AB blacklisted in the future

- **Time to promote systematic lab (AST) support to (all) Ab treatments?**
  - Critical importance of « GVP /GLP »!
  - Bacteria culturability is one challenging laboratory issue
  - True pathogenic nature of a bacterium is another one

- **Can modern technologies help?**
  - Whole Genome sequencing for *in silico* AST (expert/ref labs so far)
  - DNA micro-arrays for rapid screening of AB RES genes
  - Whole sample (microbiome) sequencing for detecting pathogens and global « RESISTOME »
  - Individual resistance profiling (whenever traditional or DNA based) still requires *strain isolation*
QUESTIONS?

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