Technews

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For Efficient Dairying

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ANTIMICROBIAL RESISTANCE (AMR)

This bulletin includes technical information based on latest developments on products, systems, techniques etc. reported in journals, companies' leaflets and books and based on studies and experience. The technical information in different issues is on different areas of plant operation. It is hoped that the information contained herein will be useful to readers.

The theme of information in this issue **Antimicrobial Resistance**. It may be understood that the information given here is by no means complete.

In this issue:

INTRODUCTION

Antimicrobial resistance is a major global public health concern and a food safety issue. Antimicrobial resistance is the ability of microbes to resist the effects of drugs that is, the germs are not killed, and their growth is not stopped. Infections with resistant organisms are difficult to treat, requiring costly and sometimes toxic alternatives. Antimicrobial resistance is one of our most serious health threats. Infections from resistant bacteria are now too common, and some pathogens have even become resistant to multiple types or classes of antibiotics.

The terms antimicrobial and antibiotic are often used interchangeably but are not synonymous. In technical terms, "antibiotics" refer only to substances of microbial origin (such as penicillin) that are active against other microbes while "antimicrobial" refers to any substance (including synthetic compounds) which destroys microbes.

BACKGROUND

In the 1940s, the widespread availability of penicillin and the subsequent discovery of streptomycin led to a dramatic reduction in illness and death from infectious diseases. However, bacteria and other disease causing organisms namely viruses, fungi, and parasites have a remarkable ability to mutate and acquire resistance genes

from other organisms and thereby develop resistance to antimicrobial drugs. When an antimicrobial drug is used, the selective pressure exerted by the drug favors the growth of organisms that are resistant to the drug's action. The extensive use of antimicrobial drugs has resulted in drug resistance that threatens to reverse the medical advances of the last seventy years.

Drug-resistant pathogens are a growing menace to all people, regardless of age, gender, or socioeconomic background. Examples of clinically important microbes that are rapidly developing resistance to available antimicrobials include bacteria that cause pneumonia, ear infections, and meningitis (e.g., *Streptococcus pneumoniae*), skin, bone, lung, and bloodstream infections (e.g., *Staphylococcus aureus*), urinary tract infections (e.g., *Escherichia coli*), foodborne infections (e.g., *Salmonella spp* or *E. coli*) acquired from milk, meat, eggs, nuts, fresh produce etc.

ANTIBIOTICS IN THE ANIMAL PRODUCTION

Antibiotics have three roles in animal production: to treat individual animals with bacterial infections, to prevent infections, and to promote growth. The first two roles are no different from uses in humans, where the drugs are used to treat and prevent infections (e.g., before major surgery, to prevent infection of the surgical site). In animals, however, antibiotics may be given to entire flocks or herds to prevent an infection from sweeping through the animal population at vulnerable points in the

production cycle, such as the weaning of young pigs from sows. Antibiotic use may be triggered by an infection in one or more animals. These prophylactic or "metaphylactic" antibiotics are usually mixed with water or food.

The third role, growth promotion, has no counterpart in human antibiotic use. It accounts for the majority of use in animals and is the focus of most legal and regulatory efforts to reduce antibiotic consumption in livestock and poultry.

ANTIMICROBIAL RESIDUES AND RESISTANCE: USAGE IN DAIRY FARMS

Mastitis is one of the most frequent infectious diseases in dairy cattle and accounts for most of the doses of antibiotics given to dairy cows. Lactating cows may be treated for clinical mastitis or to pursue bacteriological cure of a subclinical case. Antimicrobials are also used to treat other infectious diseases of dairy cows, including respiratory and uterine infections and infectious foot disease, as a result veterinary drug residues in milk represent a health risk for the consumer. The use of antimicrobials to treat dairy animals has the potential affect to human health through:

1. Increasing the risk of antimicrobial residues, and
2. Influencing the generation or selection of antimicrobial resistant foodborne pathogens.

3. Allergic reactions, toxicity, carcinogenic effects, disruption of human normal flora, provoke immunological response.

   In developed countries it has been reported that around 2%-6% of the bacterial outbreaks, in which the food vehicle is known, were related to milk and dairy products. According to study of (Ebtesham et al., 2009) it was reported that all the bacteria isolated from milk and milk products *Staphylococcus aureus*, *E coli*, *Salmonella typhi*, and *Pseudomonas aeroginosa* was found to be 68% resistance to the various antibiotics. MRSA has been found in 12% of animal products (beef, veal, lamb, pork etc.) in Denmark and Dairy products in Italy.

SUMMARY DATA**:** *Centre for Disease Control and Prevention* (CDC), US

Causative Microbes	**Illness per annum**	**Death per annum**
Carbapenem-Resistant Enterobacterioaceae	9000	600
Carbapenem-Resistant *E.coli*	7900	1400
Clostridium difficle	250000	14000
Drug–Resistant *Streptococcus pneumoniae*	1200000	7000
Methicillin-Resistant Staphylococus aureus (MRSA) , etc.	80461	11285

21,700,000 infections occurs worldwide in a year by the drug resistant Salmonella serotype typhi.

SENSITIVITY OF STARTER CULTURES TO ANTIBIOTICS

Residues in milk, resulting from the use of antibiotics in the mastitis therapy may an important cause of slowness in the acid production in fermented milk products.

Low levels of penicillin in the bulk starter milk had a more pronounced effect on acid production when the culture grown in that milk was used in cheese manufacture compared with acid production by a normal starter inoculated into cheese milk containing a high level of penicillin. For this reason, it is imperative that milk used for starter production is antibiotic free. This is one of the many reasons why so many companies use commercial frozen or freeze-dried starter cultures for direct vat inoculation rather than manufacture their own bulk starter.

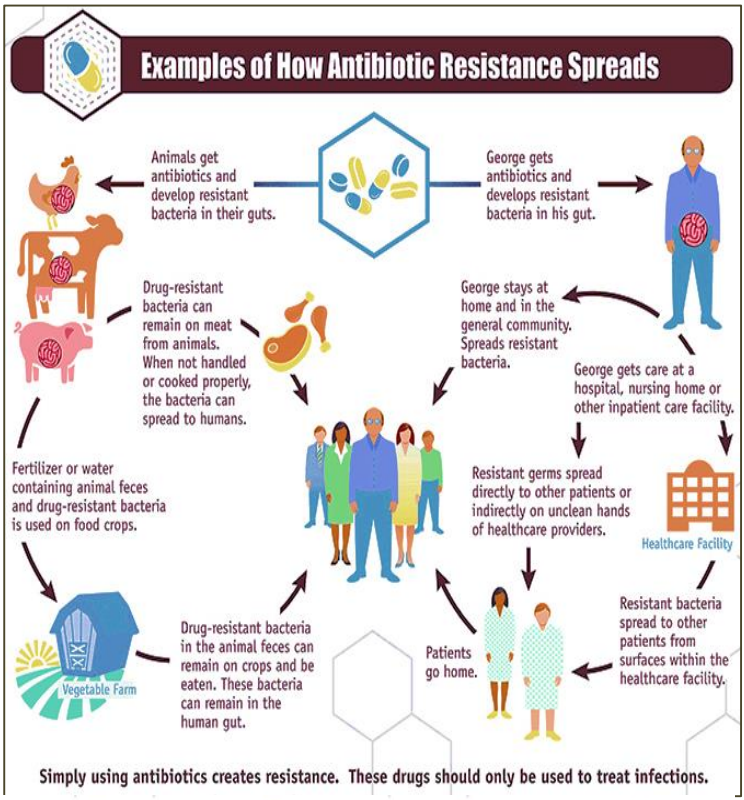
	Lactococcus lactis subsp. *Cremoris*		*Lactococcus lactis*		Mixed or Multistrain	
	Partial inhibition	Marked Inhibition (3)	Partial inhibition	Marked Inhibition (3)	Partial inhibition	Marked Inhibition (3)
Penicillin*	0.05-0.13	0.21-0.3	0.09-0.15	0.26-0.3	0.1-0.25	0.27-0.3
Tetracycline	0.11-0.16	0.3-0.4	0.09-0.21	0.28-0.65	0.09-0.20	0.29-0.35
Streptomycin	0.52-0.84	1.9-2.0	0.35-0.71	1.9-3.0	0.4-0.7	1.6-3.0
Erythromycin	-	2.0(1)	-	2.0(1)	-	-
Chloramphenicol	-	5.0(1)	-	5.0(1)	0.02-0.80	-
Chlortetracycline	0.015(1)	0.075(1)	-	5.0(1)	0.02-0.80	-
Neomycin	-	5.0(1)	5.0(1)	30.0(1)	2.5-3.5	-

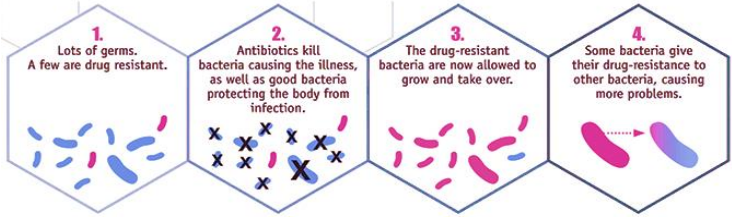
Polymyxin B*	50(1)	300(1)(2)	300(2)	-	-	-
Ampicillin	-	2.0(1)	-	2.0(1)	-	-
Novobiocin	-	5.0(1)	-	5.0(1)	-	-
Cloxacillin	1.16-2.05	2.2-4.6	1.6-2.5	3.9-5.0	1.0-2.2	3.0-4.5
Bacitracin	-	-	-	-	0.3-0.5	2.0-3.0

* Concentration expressed in International units ml. Concentration of other antibiotics in mg ml.(1) Determined using an agar diffusion method.(2) Markedly strain dependent.(3) Ranging from a reduction in acid production of 80% to complete cessation of acid production except for (1).Table taken from Haverbeck et al. (1983)

In addition, it is possible that milk may contain several different antibiotics, and that they operate synergistically to inhibit starter growth although present in low concentration.

HOW RESISTANCE HAPPENS AND SPREADS





The use of antibiotics is the single most important factor leading to antibiotic resistance around the world. Simply using antibiotics creates resistance. These drugs should only be used to manage infections.

MECHANISMS OF ANTIMICROBIAL RESISTANCE

There are a number of ways by which microorganisms are resistant to antimicrobial agents. These include:

1) The bacteria produce enzymes that either destroy the antimicrobial agent before it reaches its target or modify the drug so that it no longer is recognized by the target;

Beta-lactamases: are enzymes that hydrolyze beta-lactam drugs. As a result the cell is resistant to the action of the beta lactam drugs.

Aminoglycoside-modifying enzymes: Gram-negative bacteria may produce phosphorylating or acetylating enzymes that modify an aminoglycoside so that it is no longer active.

Chloramphenicol acetyl transferase: Gram-negative bacteria may produce an acetyl transferase that modifies chloramphenicol so that it is no longer active.

2) The cell wall becomes impermeable to the antimicrobial agent;

Gram-negative bacteria may become resistant to beta lactam antibiotics by developing permeability barriers. This usually is caused by altered porin channels in the outer membrane that no longer allow the entrance and passage of antibiotic molecules into the cell. When beta-lactams cannot reach the PBPs, the cell is resistant.

3) The target site is altered by mutation so that it no longer binds the antimicrobial agent;

• PBPs in both gram-positive and gram-negative bacteria may be altered through mutation so that beta lactams can no longer bind to them; thus the cell is resistant to these antibiotics.
• Ribosomes. Methylation of ribosomal RNA confers macrolide resistance.
• DNA gyrase and topoisomerase IV. Mutations in the chromosomal genes for DNA gyrase and topoisomerase IV confer quinolone resistance.

4) The bacteria possess an efflux pump that expels the antimicrobial agent from the cell before it can reach its target; and

A wide variety of efflux pumps provide antimicrobial resistance in both gram-positive and gram-negative bacteria. Active efflux of antibiotics is mediated by trans-membrane proteins inserted in the cytoplasmic membrane and, in the case of gram-negative organisms, in the outer membrane and the periplasm. These proteins form channels that actively export an antimicrobial agent out of the cell as fast as it enters.

5) Specific metabolic pathways in the bacteria are genetically altered so that the antimicrobial agent cannot exert an effect.

Some microorganisms develop an altered metabolic pathway that bypasses the reaction inhibited by the antimicrobial. Mutations that inactivate thymidylate synthetase block the conversion of deoxyuridylate to thymidylate. These mutants require exogenous thymine or thymidine for DNA synthesis and therefore are resistant to antagonists of the folate pathway such as the sulfonamides and trimethoprim.

RESIDUAL ANTIMICROBIAL DETECTION METHODS

1. Disk Diffusion Testing:

Objective: The agar diffusion assay is one method for quantifying the ability of antibiotics to inhibit bacterial growth. Interpretation of results from this assay relies on model-dependent analysis, which is based on the assumption that antibiotics diffuse freely in the solid nutrient medium. In many cases, this assumption may be incorrect, which leads to significant deviations of the predicted behaviour from the experiment and to inaccurate assessment of bacterial susceptibility to antibiotics. We sought a theoretical description of the agar diffusion assay that takes into consideration loss of

antibiotic during diffusion and provides higher accuracy of the MIC determined from the assay.

Analysis of agar diffusion experiments using the new model allows significantly more accurate interpretation of experimental results and determination of MICs. The model has more general validity and is applicable to analysis of other dissipative processes, for example to antigen diffusion and to calculations of substrate load in affinity purification.

The advantages of the disk diffusion method are the test simplicity that doesn't require any special equipment. This test can be done at the dairy plant without much instruments. The provision of categorical results easily interpreted by all microbiologists. This test can also be used for quantification of drugs.

2. Minimal Inhibitory Concentration Test (MIC)

The minimal inhibitory concentration (MIC) of an antimicrobial agent is the lowest (i.e. minimal) concentration of the antimicrobial agent that inhibits a given bacterial isolate from multiplying and producing visible growth in the test system. We determine the concentration in the laboratory by incubating a known quantity of bacteria with specified dilutions of the antimicrobial agent. Using NCCLS interpretive criteria the results are interpreted as susceptible, intermediate, or resistant. MIC tests can be performed using broth or agar media, but broth microdilution is the most widely used method in clinical laboratories.

3. Rapid test methods:

A. **E-Test**- is commercially available as a rapid test for MIC. E- Test strips are drug impregnated strips that contains a gradient of antibiotic on one end and ceftazidime plus clavunic acid on the other end. After inoculating the Muller Hinton agar plates with the standard inoculums using 0.5McFarland, E test strips should be placed over the plates and then the plates should be incubated over night at 37°C. After overnight incubation reading should be performed.

B. **Vitek Test**- the Vitek system is an automated system and it is based on the basic principle of photometry. The bacteria utilize a substrate which results in a colour and density change. These changes are detected by light emitting diodes and phototransistor detectors.

C. **Nucleic acid targeted detection system**- PCR, Real time PCR, DNA sequencing and Plethora of hybridization base techniques offer rapid and sensitive methods to detect the presence of resistant genes and crucial in the elucidation of resistance mechanism

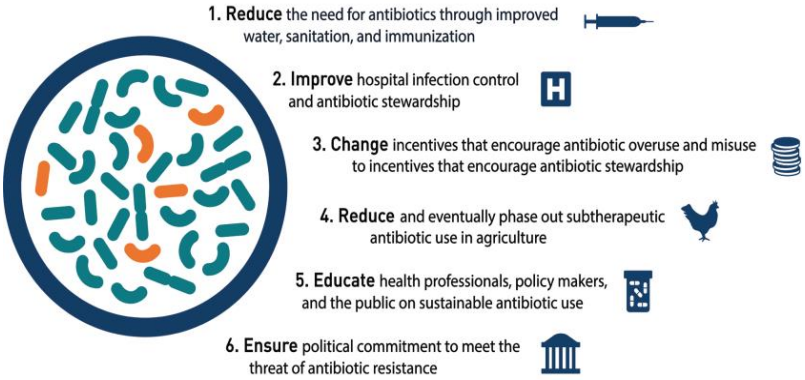
4. The other test methods are tabulated below:

Antibiotic	Make	Principle	Test time
β-lactam antibiotics	Lumac Rapid Antibiotic test, Lumac by,	ATP Measurement	90 min
Wide range of antibiotics	Valio T-102 test, Valio Ltd.	Bacterial growth inhibition	4-30 min
β-lactam antibiotics	Delvotest accelerator, DSM-Food Specialties	Bacterial growth inhibition	105-140 min
β-lactam antibiotics	Delvo-X–Press, Food Specialties	Receptor enzyme assay	7 min
β-lactam antibiotics	Delvotest BLF, DSM-Food Specialties	Bacterial growth inhibition	5 min
β-lactam, Tetracyclines, sulphonamides, Macrolides, Aminoglucosides & Fluroquinolones	Delcotest SP-NT, DSM-Food Specialties	Bacterial growth inhibition	180 min
β-lactam antibiotics	Penzyme test, UCB-Bioproducts s.a.	Enzymatic, colorimetric assay	20 min
β-lactam antibiotics	βeta-s.t.a.r. Neogen Corporation	-	5 min
β-lactam antibiotics	Charm MRL Beta-lactam Test (ROSA), ROSA SL3 Test for Beta-Lactam Drugs	Lateral Flow-Rapid receptor assay utilizing ROSA (Rapid One Step Assay)	8 & 3 min

	Charm Sciences Inc.	technology. 2& 3 line reaction	
Sulfamethazine, Sulfadimethoxine	ROSA SDSM Test	Lateral Flow. Rapid receptor/immunoassay utilizing ROSA (Rapid One Step Assay) technology. 3-line reaction	8 min
β-lactam antibiotics	Immunoassays Lactek, Idexx laboratories, Inc.	Immunoassay	8 min
Beta lactams SDM, SMZ Tetracyclines Neomycin Streptomycin Quinolones	Parallux, Idexx Laboratories, Inc.	Competitive EIA method	5 min
β-Lactum & Tetracycline	en βeta-s.t.a.r. Combo 3.0, Neogen Corporation	-	3 min
	βeta-s.t.a.r. 1+1, Neogen Corporation	-	2 min
Aminoglycosides, Amphenicols/Chloramphenicol, Beta-lactams, Macrolides/Lincosamides, Novobiocin, Sulfonamides & Tetracyclines	Charm II Kits on MRL & US safe level Charm Sciences Inc.	Microbial Receptor Assay	10 min (Depends on drug family)
β-Lactum & Tetracycline	TwinSensor Milk &	Receptor assays in dipstick	3-6 min

	Twin Express Milk (Unisensor s.a.),	format	
Beta lactams Tetracyclines Sulfamethazine Gentamicin	SNAP Test Kits - IDEXX Laboratories, Inc.	Enzyme-linked receptor-binding assay	10 min
β-Lactum, Tetracycline and sulphonamide	Generic Trisensor (Unisensor s.a.)	Competitive test involving two receptors and generic monoclonal antibodies in one single operation.	6 min
β-Lactum, Tetracycline, streptomycine and chloramphenicol residues	4Sensor Milk (Unisensor s.a.) or the seta-s.t.a.r. 4D (Neogen Corporation)	Receptor assays in dipstick format	10 min
Chloramphenicol, tetracyclines, spectinomycin, Sulfamethazine, quinolones and Gentamicin	Quicking Biotech Co.,Ltd	Competitive lateral flow immunochromatographic assay	5-10 min

STATERGIES NEEDED FOR CONTROLLING USE OF ANTIBIOTICS



TO FIGHT RESISTANCE

1. **Preventing Infections, Preventing the Spread of Resistance** – Avoiding infections in the first place reduces the amount of antibiotics that have to be used and reduces the likelihood that resistance will develop during the therapy. There are many ways that drug- resistant infections can be prevented: immunisation, safe food preparation, hand washing

and using antibiotics as directed and only when necessary.

2. **Tracking –** to gather data on antibiotic resistant infections, causes of infections and whether there are particular reasons (risk factors) that caused some people to get a resistant infection. With that information, experts can develop specific strategies to prevent those infections and prevent the resistant bacteria from spreading.

3. **Improving Antibiotic Prescribing**: Perhaps the single most important action needed to greatly slow down the development and spread of antibiotic-resistant infections is to change the way antibiotics are used. Up to half of antibiotic used in humans and animals is unnecessary and inappropriate and makes everyone less safe. Stopping even some of inappropriate and unnecessary use of antibiotics would greatly help in slowing down the spread of resistant bacteria. "Always to use right antibiotics and to administer them in right way in every use".

4. **Developing New Drugs and Diagnostic Tests:** Because antibiotic resistance occurs as a part of a natural process which bacteria evolve, it can be slowed but not stopped. Therefore, we will always need new antibiotics to keep up with resistant bacteria as well as a new diagnostic tests to track the development of resistance.

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